JOURNAL OF AGRICULTURAL RESEARCH

VOLUME 57

JULY 1–DECEMBER 15, 1938



ISSUED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE
WITH THE COOPERATION OF THE ASSOCIATION OF
LAND-GRANT COLLEGES AND UNIVERSITIES

UNITED STATES
GOVERNMENT PRINTING OFFICE
WASHINGTON : 1939

JOINT COMMITTEE ON POLICY AND MANUSCRIPTS

FOR THE UNITED STATES DEPARTMENT OF AGRICULTURE

H. G. KNIGHT, CHAIRMAN
Chief, Bureau of Chemistry and Soils

JOHN W. ROBERTS
Principal Pathologist, Bureau of Plant Industry

BENJAMIN SCHWARTZ
Principal Zoologist, Chief, Zoological Division, Bureau of Animal Industry

FOR THE ASSOCIATION OF LAND-GRANT COLLEGES AND UNIVERSITIES

S. W. FLETCHER
Director of Research, Pennsylvania Agricultural Experiment Station

L. E. CALL
Director, Kansas Agricultural Experiment Station

C. E. LADD
Director, New York (Cornell) Agricultural Experiment Station

EDITORIAL SUPERVISION

M. C. MERRILL
Chief of Publications, United States Department of Agriculture

Articles for publication in the Journal must bear the formal approval of the chief of the department bureau, or of the director of the experiment station from which the paper emanates. Each manuscript must be accompanied by a statement that it has been read and approved by one or more persons (named) familiar with the subject. The data as represented by tables, graphs, summaries, and conclusions must be approved from the statistical viewpoint by someone (named) competent to judge. All computations should be verified.

Station manuscripts and correspondence concerning them should be addressed to S. W. Fletcher, Director of Research, Pennsylvania Agricultural Experiment Station, State College, Pa.

Published on the 1st and 15th of each month. This volume will consist of 12 numbers and the contents and index.

Subscription price:

Entire Journal:	Domestic, $3.25 a year (2 volumes).
	Foreign, $4.75 a year (2 volumes).
Single numbers:	Domestic, 15 cents.
	Foreign, 20 cents.

Articles appearing in the Journal are printed separately and can be obtained by purchase at 5 cents a copy domestic; 8 cents foreign. If separates are desired in quantity, they should be ordered at the time the manuscript is sent to the printer. Address all correspondence regarding subscriptions and purchase of numbers and separates to the Superintendent of Documents, Government Printing Office, Washington, D. C.

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 57 WASHINGTON, D. C. JULY 1–DECEMBER 15, 1938

CONTENTS

Page

Effect of Different Soil Colloids and Whole Soils on the Toxicity of Sodium Selenate to Millet. PHILIP L. GILE, HUBERT W. LAKIN, and HORACE G. BYERS. Illus. 1

Growth Substance and the Development of Crown Gall. S. B. LOCKE, A. J. RIKER, and B. M. DUGGAR. Illus. 21

A Photoelectric Sorter for Length Measurements of Fibers of Seed Cotton. BURT JOHNSON. Illus. 41

Variability in Germination of Freshly Harvested *Avena*. F. A. COFFMAN and T. R. STANTON. 57

Two Distinct Strains of the Nematode *Aphelenchoides fragariae* Occuring on Strawberry Plants in the United States. J. R. CHRISTIE. Illus. 73

Preservation of Cyanogenetic Plants for Chemical Analysis. REINHOLD R. BRIESE and JAMES F. COUCH. 81

Relation of Atmospheric Conditions to Enlargement Rate and Periodicity of Winesap Apples. C. P. HARLEY and M. P. MASURE. Illus. 109

The Solubility of Casein in Salts of Certain Organic Acids, and Its Fractionation by Means of These Acids. RALPH H. MCKEE and STEPHEN P. GOULD. 125

Relation of Bitterness to the Toxic Principle in Sweetclover. WILLIAM K. SMITH and R. A. BRINK. 145

Cytology of Twin Cotton Plants. J. M. WEBBER. Illus. 155

Moisture Movement in Wood Above the Fiber-Saturation Point. LOUIS W. REES and STANLEY J. BUCKMAN. Illus. 161

The Effect of Natural Selection in a Mixture of Barley Varieties. H. V. HARLAN and M. L. MARTINI. Illus. 189

Effect of Neutral Salts of Sodium and Calcium on Carbon and Nitrogen of Soils. V. P. SOKOLOFF. 201

An Apparent Induced Loss of Nitrogen-Fixing Ability in *Azotobacter*. CHARLES R. STUMBO and P. L. GAINEY. Illus. 217

The Reactions of Wireworms to Arsenicals. CHARLES E. WOODWORTH. 229

Rate of Spread of Surface Fires in the Ponderosa Pine Type of California. JOHN R. CURRY and WALLACE L. FONS. Illus. 239

Anatomy of the Leaf and Stem of *Gossypium*. IRMA E. WEBBER. Illus. 269

Two Mastigosporium Leaf Spots on Gramineae. RODERICK SPRAGUE. Illus. 287

Preliminary Biochemical Studies on Effects of Certain Environmental Factors on Development and Composition of the Peanut. SAMUEL L. JODIDI. 301

Inflorescence Blight of the Date Palm. J. G. BROWN and KARL D. BUTLER. Illus. 313

Experiments on the Transmission of Potato Viruses by Vectors. T. P. DYKSTRA and W. C. WHITAKER. Illus. 319

Vertical Migration, Distribution, and Survival of Infective Horse Strongyle Larvae Developing in Feces Buried in Different Soils. JOHN T. LUCKER. Illus. 335

Effect of Infestation With the Nematode *Cooperia curticei* on the Nutrition of Lambs. JOHN S. ANDREWS. Illus. 349

A Method of Determining Root Knot Resistance in Beans and Cowpeas in the Seedling Stage. KEITH C. BARRONS. Illus. 363

Hood and Supernumerary Spike Development in Barley. O. T. BONNETT. Illus. 371

Influence of Japanese Beetle Instar on the Sex and Population of the Parasite *Tiphia popilliavora*. M. H. BRUNSON. 379

Microchemical Studies of Potato Tubers Affected With Blue Stem Disease. L. M. HILL and C. R. ORTON. Illus. 387

A Comparison of the Vitamin D Potency of the Stemmy and Leafy Portions of Alfalfa Hay. G. C. WALLIS
Foliar Diagnosis in Relation to Development and Fertilizer Treatment of the Potato. WALTER THOMAS and WARREN B. MACK. Illus
Observations on Muskrat Damage to Corn and Other Crops in Central Iowa. PAUL L. ERRINGTON
Toxic and Repellent Properties of Some Inorganic and Organic Compounds and Mixtures in Grasshopper Baits. CHARLES H. RICHARDSON and EDWIN J. SEIFERLE
Proteolytic Activity in Relation to the Blackening of Potatoes After Cooking. A. FRANK ROSS and W. E. TOTTINGHAM. Illus
Morphology of the Digestive Tract of the Blackfly (*Simulium nigroparvum*). JAMES A. COX. Illus
Seasonal Variations in the Carbohydrate and Nitrogen Content of Roots of Bearing Pecan Trees. C. L. SMITH and J. G. WAUGH. Illus
Applicability of Nutrient-Solution Purification to the Study of Trace-Element Requirements of Rhizobium and Azotobacter. ROBERT A. STEINBERG
Digestible Nutrients and Metabolizable Energy in Certain Silages, Hays, and Mixed Rations. F. W. CHRISTENSEN and T. H. HOPPER
The Relation of Color and Carotene Content of Roughage in the Dairy Ration to the Color, Carotene Content, and Vitamin A Activity of Butterfat. R. E. HODGSON, J. C. KNOTT, H. K. MURER, and R. R. GRAVES. Illus
The Effect of Environmental Temperature on Mortality, Rate of Growth, and Utilization of Food Energy in White Leghorn Chicks. C. F. WINCHESTER and M. KLEIBER. Illus
Some Physiological Studies of Crown Gall and Contiguous Tissue. R. NAGY, A. J. RIKER, and W. H. PETERSON. Illus
Studies on Probable Damage by Blister Rust in Some Representative Stands of Young Western White Pine. THOMAS W. CHILDS and J. W. KIMMEY. Illus
The Essentiality of Gallium to Growth and Reproduction of *Aspergillus niger*. ROBERT A. STEINBERG
Time Interval Between Clutches in Rhode Island Red Pullets. F. A. HAYS. Illus
Relation Between Fiber Length and Maturity in Cotton. R. S. HAWKINS. Illus
A Mosaic Disease of Turnip. C. M. TOMPKINS. Illus
Assays of Riboflavin and the Filtrate Factor in Certain Milk Products. T. H. JUKES and G. A. RICHARDSON
Relation of Soil Temperature to Chlorosis of Gardenia. LINUS H. JONES. Illus
Resistance in the Red Raspberry to the Mosaic Vector *Amphorophora rubi* Kalt. GLENN A. HUBER and C. D. SCHWARTZE. Illus
The Carotene Content of Market Hays and Corn Silage. HERBERT G. WISEMAN, EDWARD A. KANE, LEO A. SHINN, and C. A. CARY. Illus
Host-Parasite Relations in Red Clover Plants Resistant and Susceptible to Powdery Mildew, *Erysiphe polygoni*. OLIVER F. SMITH. Illus
Differentiation of Wood-Decaying Fungi by Their Reactions on Gallic or Tannic Acid Medium. ROSS W. DAVIDSON, W. A. CAMPBELL, and DOROTHY J. BLAISDELL. Illus
An Analysis of the Blood of the Sixth-Instar Southern Armyworm (*Prodenia eridania*). FRANK H. BABERS
The Biological Disposition of Rotenone After Ingestion by the Southern Armyworm. P. A. WOKE
Sampling Error in Timber Surveys. A. A. HASEL. Illus
A Study of Protein Extract From Soybeans With Reference to Its Use in Food. SYBIL WOODRUFF, EVELYN CHAMBERS, and HELEN KLAAS. Illus
Linkage of the Q B Gs Group in Sorghum. J. C. STEPHENS and J. R. QUINBY. Illus
Further Investigations of Brown-Staining Fungi Associated With Engraver Beetles (*Scolytus*) in White Fir. ERNEST WRIGHT. Illus
Complete or Partial Inhibition of Flowering in Certain Plants When Days Are Too Short or Too Long. H. A. ALLARD. Illus

Page

Stagonospora Leaf Spot and Root Rot of Forage Legumes. FRED REUEL JONES and J. L. WEIMER. Illus. 791
Histology of Apple Fruit Tissue in Relation to Cracking. LEIF VERNER. Illus. 813
Physiological Conditions Which Produce Wing Development in the Pea Aphid. CARL W. SCHAEFER. Illus. 825
The Growth Curve of Sorghum. A. T. BARTEL and J. H. MARTIN. Illus. 843
Correlations Between Biological Essentiality and Atomic Structure of the Chemical Elements. ROBERT A. STEINBERG. 851
Laboratory Studies on *Tetrastichus xanthomelaenae* Rond. and *Tetrastichus* sp., Two Hymenopterous Egg Parasites of the Elm Leaf Beetle. P. A. BERRY. Illus. 859
Effect of Exterior Temperature Upon Press Fluid, Shear Force, and Cooking Losses of Roasted Beef and Pork Muscles. ALICE M. CHILD and MARY J. SATORIUS. Illus. 865
Respiration and Lactic Acid Production by a Fungus of the Genus *Rhizopus*. SELMAN A. WAKSMAN and J. W. FOSTER. Illus. 873
Determination and Occurrence of Boron in Natural Phosphates, Superphosphate, and Defluorinated Phosphate Rocks. LEWIS F. RADER, JR., and W. L. HILL. Illus. 901
The Anatomical Development of *Lepidium draba*. AUSTIN O. SIMONDS. Illus. 917
Black Ring, a Virus Disease of Cabbage and Other Crucifers. C. M. TOMPKINS, M. W. GARDNER, and H. REX THOMAS. Illus. 929
Soil-Fertility Standards for Growing Northern Conifers in Forest Nurseries. S. A. WILDE. 945

ERRATA AND AUTHORS' EMENDATION

Page 5, table 1, column 4, "P_2O_1" should be "P_2O_5."

Page 6, table 2, column 3, "$Al_2\ O_3\ K\ Fe_2\ O_3$" should be "$Al_2\ O_3 + Fe_2\ O_3$."

Page 116, second line from bottom, after the word "growth" the remainder of the paragraph should read: "as does evaporating power. Mean atmospheric temperatures, on the other hand, seem to be somewhat negatively correlated with growth in the last two periods, but not in the first."

Page 118, line 11, delete the word "evaporation."

Page 149, tenth line from bottom of text, "microscopically" should be "macroscopically."

Page 164, tenth line from bottom, insert "g" after "0.01."

Page 168, table 2, line 2, column 11, change "0.00341" to "0.00314."

Page 173, sixth line from bottom, "sapwood" should be "heartwood," and seventh line from bottom, "in" should be "from" and "heartwood" should be "sapwood."

Page 347, line 15, the first "152 days" should be "126 days."

Page 356, table 5, column 8, heading "Nitrogen free" should be "Nitrogen free extract."

Page 357, table 7, column 7, "−26.74" should be "+26.74."

Page 398, table 1, column 2, under "Plot No.," the 18th number, "4," should be "14;" the 20th number "8", should be "10;" and the next to last number, "10," should be "16."

Page 403, "figure 1" should be "figure 2," and page 411, "figure 2" should be "figure 1."

Page 410, table 6, next to last word in heading, "dry-weather" should be "dry-weight."

Page 426, table 1, last column, "100" appearing beneath "percent" should align with "22" appearing below "Hours" and all succeeding figures through "93" should be correspondingly aligned. The first "0" after "93" should be deleted.

Page 455, line 4, delete the word "below."

Page 463, eighth line from bottom, "2 to 4 million bacteria" should read "10 to 20 million bacteria."

Page 468, line 11, "465" should be "467."

Page 482, line 1, "1924" should be "1923."

Page 484, table 2, column 7, next to last line, "35.55" should be "36.55."

Page 489, table 4, column 8, line 5, "1.998" should be "1.988;" column 6, ninth line from bottom, "21.83" should be "2.183;" and column 7, sixteenth line from bottom, "4.22" should be "0.422."

Page 491, table 4, column 7, line 3, "2.36" should be "0.236."

Page 493, the second line in paragraph 2 should be inserted between the first and second line in paragraph 1.

Page 496, table 5, next to the last column, line 7, "7.864" should be "78.64."

Page 503, table 7, last column, line 8, "29.6" should be "29.7."

Page 504, table 8, last column, line 4, "41.8" should be "14.8;" "Trial 8" should be "Trial 13," and "Trial 17" should be "Trial 16;" and column 3, line 3, "48.5" should be "43.9."

Page 507, table 10, last column, line 6, "+0308" should be "−.0308."

Page 509, table 11, "Trial 19" should be "Trial 21."

Page 531, line 5, insert "See" before "Almquist."

Page 543, line 15, $\frac{\text{"food}-\text{excreta}+100\text{"}}{\text{food}}$ should be $\frac{\text{"food}-\text{excreta }100\text{"}}{\text{food}}$

Page 570, table 1, tenth line from bottom of table, column 2, "44.36" should be "443.6."

Page 603, line 2, "chicks [3]" should be "chicks (3) [3]."

Page 606, last line, "Diet 96 (7)" should be "Diet 96 (4)."

Page 625, the positions of the illustrations in figures 1 and 2 should be reversed.

Page 640, line 5, "0.195" should be "0.1915."

Page 642, line 5 of text, "0.5" should be "0.05."

Page 686, second line from bottom, columns 4 and 6, only one minus sign should be used.

Page 687, line 9, columns 4 and 6, only one minus sign should be used.

Page 733, table 10, first column, third line from bottom, "20 by 70" should be "20 by 80."

Page 738, plate 1, the material in "*A*" should be "*B*," and that in "*B*" should be "*A*."

Page 753, table 3, column 2, next to last line of factors, "gsgs" should be "Gsgs."

Page 818, line 5, "plate 2, A" should be "plate 5, B."

Page 868, table 1, column 1, second line from bottom, insert "t" at the beginning of the line.

Page 876, table 2, column 1, lines 1 and 2, insert "3" and "4," respectively, in place of leaders.

Page 917, under heading, Materials and Methods, line 16, insert "95 percent" between "48 cc of" and "alcohol."

Page 946, table 3, column 5, insert "Pounds" above figures.

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 57 WASHINGTON, D. C., JULY 1, 1938 No. 1

EFFECT OF DIFFERENT SOIL COLLOIDS AND WHOLE SOILS ON THE TOXICITY OF SODIUM SELENATE TO MILLET[1]

By PHILIP L. GILE, *senior chemist*, HUBERT W. LAKIN, *assistant chemist*, and HORACE G. BYERS, *principal chemist, Soil Chemistry and Physics Research Division, Bureau of Chemistry and Soils, United States Department of Agriculture*

INTRODUCTION

Investigations of the occurrence of selenium in soils, shales, waters, and vegetation in the United States have been reported by Byers (*2, 3*).[2] In the course of these investigations it became important to know whether the toxicity of selenium compounds to plants varies with the character of the soil. Previous studies by Hurd-Karrer (*8, 9*) indicate that this is the case, since she found that the amount of selenium absorbed by the plant and its injurious effect are markedly influenced by the sulphate available in the soil. She also found that the toxicity of sodium selenate varied with other characteristics of the soil, since sodium selenate was more injurious to wheat in Pierre clay than in Keyport clay loam (*9*). No systematic study, however, has been made of the toxicity of selenium compounds in different types of soil.

The results reported in this paper deal with the effects of different soil colloids and of different whole soils on the toxicity of sodium selenate to millet. The effects of the colloids on the absorption of selenium from the selenate applications are also considered. It should be emphasized that this study concerns only selenium applied as sodium selenate, since work in progress shows that quite different results are obtained when selenium is applied as sodium selenite.

METHODS

The method of investigation first adopted was the same as that followed in a previous study, which dealt with the effects of soil colloids on the toxicity of calcium arsenate (*6*). Foxtail millet (*Setaria italica* (L.) Beauv.) was grown in quartz sand with which sufficient soil was mixed to give a soil-colloid content of approximately 1 percent. Different quantities of sodium selenate were applied. The quantity of selenium required to reduce the yield one-half was determined for each mixture from a graph of yields plotted against selenate applications. This half-toxicity value for the sand-soil mixture was compared with the half-toxicity value of a parallel, pure quartz-sand series, and the difference between the two values was taken as the effect of the soil colloid on the toxicity of sodium selenate. The advantages of this method and the objections to it have been discussed in previous publications (*5, p. 4; 6, p. 478*).

[1] Received for publication August 24, 1937; issued July 1, 1938.
[2] Italic numbers in parentheses refer to Literature Cited, p. 19.

Each pot, filled with pure quartz sand or with a sand-soil mixture, received 0.93 g of potassium nitrate, 0.33 g of ammonium sulphate, 0.0185 g of ferric tartrate, 0.42 g of magnesium chloride ($MgCl_2.6H_2O$), and 0.0015 g of manganese sulphate ($MnSO_4.2H_2O$). From 0.27 to 0.94 g of monocalcium phosphate per pot was supplied, the quantity being varied according to the capacity of the soil for rendering phosphate unavailable to millet.

Essentially the same method was used in the experiments with whole soils.

Glazed earthenware pots holding about 5,000 g of quartz sand were used as containers. They were kept in a greenhouse, and the moisture content was maintained at 15 percent with distilled water. Ten millet plants per pot were grown for periods ranging from 18 to 35 days, according to the season. The plants were forming joints when cut. Only the parts of the plant above ground were harvested. Most of these were later analyzed for selenium by the method described by Williams and Lakin (*17*). Two experiments were also conducted with Marquis wheat (*Triticum aestivum* L.) grown for 48 days, and one experiment was conducted with White London mustard (*Brassica alba* (L.) Boiss.) grown for 26 days.

All except two of the soils used in these experiments were parts of samples utilized in former studies of soil colloids. Data regarding them are given in the following Department of Agriculture publications: Nos. 190, 195, 298, and 300 in Department Bulletins 1193 and 1311 (*7*, *14*); Nos. 6678, 6679, 6718, 6719, 6842, 6977, 8736, and 8737 in Technical Bulletin 316 (*11*); Nos. B–407 and 9475 in Technical Bulletin 430 (*12*); No. 9804 in Technical Bulletin 484 (*4*); and No. B–1086 in Technical Bulletin 502 (*1*). The Keyport clay loam was a sample of surface soil from the Arlington Experiment Farm of the Department of Agriculture at Arlington, Va., and the Clarion loam was a surface soil from Ames, Iowa. These two soils have not been analyzed. Selenium in some form has been found in most of the soils (*15*), but none of them contain selenium sufficiently available to millet to impart more than 1 part per million to the dry substance of the plant.

EXPERIMENTS CONDUCTED WITH QUARTZ SAND AND SAND-SOIL MIXTURES

Some preliminary tests were made to determine the amount of selenium that would reduce the yield of millet to about one-half that of the checks. The experiments reported in table 1 were then conducted. The medium for growth was in all cases either pure quartz sand or a sand-soil mixture containing approximately 1 percent of soil colloid. Table 1 contains data for calculating the quantities of selenium required to reduce the yield of millet one-half in seven different sand-soil mixtures. It also contains data showing the manner in which increasing quantities of selenate decrease the yield, and the effects of phosphate and sulphate on the toxicity of the selenate.

The manner in which the yield decreases with increasing quantities of sodium selenate is shown in figure 1. The curves in this figure represent averages of results obtained in quartz sand and in different sand-soil mixtures in experiments 1, 2, 4, 5, and 6, table 1. Percentage yields rather than actual yields are given in this graph, the

weight of plants grown without selenium being taken as 100. The use of relative values facilitates averaging the widely different actual yields obtained in the different experiments. It is apparent from the form of the curve that the quantities of selenium required to reduce the yield to between 60 and 15 percent of normal can be determined with considerable accuracy.

Incidentally, it may be noted that this curve for the toxicity of sodium selenate has the same form as the curve obtained for the toxicity of calcium arsenate (*6*). Both toxicity curves are similar to Mitscherlich's (*13*) curve for increased growth with increase in the minimum factor in that the toxicity curves are Mitscherlich's curve turned upside down.

These results are sufficient to show that soluble sulphates should be kept approximately constant in comparing the effects of different

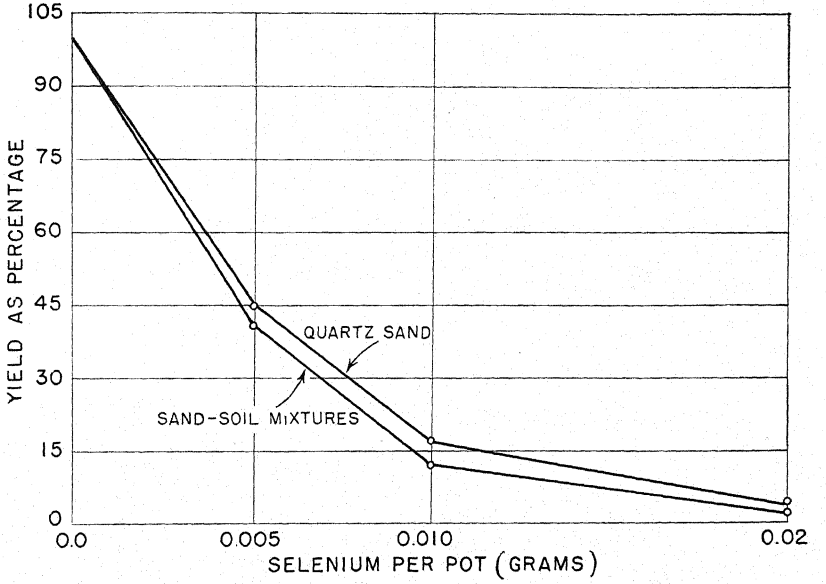


FIGURE 1.—Effect of increasing quantities of sodium selenate on yield of millet. Yield with no silicate=100.

soils on selenate toxicity. In the experiments on the effects of the soil colloids the available sulphate applied was 0.24 g of SO_4 per pot unless otherwise noted. This quantity was largely in excess of the crop's requirements, since in experiment 6, wherein the influence of sulphate application was tested, the plants receiving no selenium gave approximately the same yield with 0.024 g of SO_4 as with the usual 0.24 g. This quantity is also large as compared with the quantities of soluble SO_4 naturally present in the soils added to the quartz sand in different experiments. Small variations obtaining in different experiments at this comparatively high level of available SO_4 would not be expected to affect appreciably the half-toxicity values, since a threefold increase in SO_4 in experiment 1 (the excess sulphate occurring in the superphosphate application) increased the half-toxicity figure by only about 60 percent and a 90-percent decrease in SO_4 in experiment 6 only halved the toxicity figure.

TABLE 1.—*Effect of different soil colloids on the toxicity of sodium selenate to plant growth*

[All soils added to quartz sand in sufficient quantity to supply 1 percent of colloid; millet grown except as otherwise indicated]

EXPERIMENT 1 (OCT. 15 TO NOV. 17)

Laboratory no.	Kind of soil mixed with quartz sand	Depth	P_2O_5 applied per pot	Sodium selenate added per pot, expressed, as Se	Air-dry yield of plants per pot				Selenium in air-day plants	Half-toxicity value (Se required per pot to reduce yield one-half)
					Series A	Series B	Series C	Average		
		Inches	*Gram*	*Gram*	*Grams*	*Grams*	*Grams*	*Grams*	*P. p. m.*	*Gram*
	Quartz sand only		0.20	0.000	3.00	2.40	3.87	3.09	0	0.0047
				.005	1.41	1.21	1.83	1.48	400	
				.010	.44	.83	.56	.61	690	
				.020	.07	.14	.12	.11	1,500	
190	Wabash silt loam	15–36	.20	.000	5.12	4.92	5.18	5.08	0	[1] .0057
				.010	.51	.45	.37	.44	1,300	
				.020	.06	.09	.08	.08	1,900	
9804	Columbiana clay	0–10	.50	.000	5.19	4.13	4.99	4.77	0	[1] .0053
				.010	.44	.48	.43	.45	1,100	
				.020	.02	.05	.06	.04		
9804	do	0–10	[2] .50	.000	4.02	4.72	4.42	4.39	0	[2] .0080
				.010	1.43	1.36	2.18	1.66	400	
				.020	.41	.36	.39	.39	1,000	

[1] This value is a little higher than the true value because of the curvature of the toxicity curve (see fig. 1) and interpolation between distant points.
[2] Superphosphate instead of monocalcium phosphate.

EXPERIMENT 2 (FEB. 11 TO MAR. 18)

	Quartz sand only		0.15	0.000	3.69	2.88	2.79	3.12		0.0054
				.005	1.87	1.50	1.71	1.69		
				.010	.48	.56	.48	.51		
298	Manor loam	7–20	.40	.000	5.14	5.53	5.15	5.27		.0044
				.005	2.13	2.16	2.43	2.24		
				.010	.35	.68	.63	.55		
195	Clarksville silt loam	0–10	.25	.000	5.14	3.99	4.66	4.60		.0047
				.005	2.04	2.32	2.15	2.17		
				.010	.60	.80	.78	.73		
8737	Marshall silt loam	13–24	.25	.000	5.15	4.60	4.92	4.89		.0048
				.005	1.88	2.65	2.28	2.27		
				.010	.77	.59	.66	.67		

EXPERIMENT 3 (JAN. 14 TO FEB. 25)

	Quartz sand only		0.10	0.000	2.48	2.91		2.70		0.0060
				.007	1.21	1.03		1.12		
	do		.20	.000	3.32	3.87		3.60		.0043
				.007	.67	.61		.64		
	do		.30	.000	3.76	3.54		3.65		.0044
				.007	.73	.76		.75		

EXPERIMENT 4 (APR. 1 TO MAY 1)

	Quartz sand only		0.225	0.000	3.47	1.65	3.59	2.90		0.0038
				.005	1.00	.48	1.50	.99	400	
				.010	.37	.50	.49	.45		
	do		.45	.000	3.45	2.90	3.14	3.16		.0046
				.005	.64	.94	1.12	.90		
				.010	.26	.28	.38	.31		

TABLE 1.—*Effect of different soil colloids on the toxicity of sodium selenate to plant growth*—Continued

EXPERIMENT 5 (JUNE 20 TO JULY 8)

Laboratory no.	Kind of soil mixed with quartz sand	Depth	P_2O_5 applied per pot	Sodium selenate added per pot, expressed, as Se	Air-dry yield of plants per pot				Selenium in air-day plants	Half-toxicity value (Se required per pot to reduce yield one-half)
					Series A	Series B	Series C	Average		
		Inches	*Gram*	*Gram*	*Grams*	*Grams*	*Grams*	*Grams*	*P. p. m.*	*Gram*
	Quartz sand only		0.15	0.000	2.10	2.54	2.19	2.28		0.0043
				.005	.92	1.04	.89	.95	590	
				.010	.34	.37	.33	.35		
8736	Marshall silt loam	0–13	.15	.000	5.42	5.33	5.42	5.39		.0038
				.005	1.81	1.67	2.11	1.86		
6977	Cecil sandy clay loam	0–6	.40	.000	4.40	3.38	4.14	3.97		.0037
				.005	1.21	1.46	1.39	1.35		
	EXPERIMENT 6 (OCT. 11 TO NOV. 15), THE USUAL 0.24 G SO_4 PER POT ADDED									
	Quartz sand only		0.20	0.000	1.76	1.45	1.63	1.61		0.0046
				.005	.54	.84	.80	.73	550	
8736	Marshall silt loam	0–13	.20	.000	2.10	2.08	2.30	2.16		.0043
				.005	.76	.97	.98	.90	790	
6977	Cecil sandy clay loam	0–6	.53	.000	1.98	2.22	2.33	2.18		.0041
				.005	.92	.74	.82	.83	740	
	EXPERIMENT 6 (OCT. 11 TO NOV. 15), ONLY 0.024 G SO_4 PER POT ADDED									
	Quartz sand only		0.20	0.000	1.40	1.32	1.27	1.33		0.0025
				.005	.01	.01	.04	.02		
8736	Marshall silt loam	0–13	.20	.000	2.29	2.23	2.30	2.27		.0025
				.005	.01	.01	.01	.01		
6977	Cecil sandy clay loam	0–6	.53	.000	1.94	2.22	2.12	2.12		.0025
				.005	.01	.03	.01	.02		
	EXPERIMENT 7 (NOV. 27 TO JAN. 14), WHEAT GROWN INSTEAD OF MILLET									
	Quartz sand only		0.15	0.000	2.92	3.32	3.24	3.16	(1)	0.0072
				.010	.82	.96	1.08	.95	1,700	
				.020	.27	.28	.20	.25	2,500	
6977	Cecil sandy clay loam	0–6	.40	.000	3.07	3.30	3.80	3.39	(1)	.0070
				.010	.99	1.00	.88	.96	1,700	
				.020	.15	.22	.25	.21	3,200	
	EXPERIMENT 8 (APR. 3 TO APR. 29), MUSTARD GROWN INSTEAD OF MILLET									
	Quartz sand only		0.20	0.000	1.98	1.31	1.94	1.74		0.0051
				.005	.87	.95	.86	.89		
				.010	.64	.49	.43	.52		
				.020	.22	.08	.20	.17		

The effects of soil colloids on the toxicity of sodium selenate are shown in table 2, which summarizes experimental results given in table 1. All data in the table pertain to millet except those in the last line, which apply to wheat. It will be seen that additions of widely different soils to quartz sand, up to 1 percent of colloidal matter, in no case diminish the toxicity of sodium selenate. In fact, the quantity of selenate producing one-half injury is slightly less in the sand-soil mixtures than in pure quartz sand in every case except the two noted as not accurately determined. Evidently, then, within the limits of error inherent in the method, soil colloids have no effect

on the availability of sodium selenate. This conclusion is based on the results of only seven soils, but the colloids of these soils have silica-sesquioxide ratios ranging from 3.33 to 0.81. Moreover, the effects produced by these same soils on superphosphate (*5*) and on calcium arsenate in previous work (*6*) covered pretty well the range of effects produced by a much greater number of soils.

TABLE 2.—*Toxicity to millet of sodium selenate in sand-soil mixtures containing 1 percent of soil colloid*

Experiment No.	Kind of soil mixed with quartz sand	Molecular ratio of SiO_2 to Al_2O_3K Fe_2O_3 of colloidal material	Half-toxicity value (Se required per pot to yield one-half)		Se in plants (calculated for application of 0.005 g Se per pot) [1]	
			In sand-soil mixture	In quartz sand	In sand-soil mixture	In quartz sand
			Gram	*Gram*	*P. p. m.*	*P. p. m.*
1	Wabash, No. 190	3.33	[1] 0.0057	0.0047	650	400
5	Marshall, No. 8736	2.91	.0038	.0043	----------	----------
6	----do	2.91	.0043	.0046	790	550
2	Marshall, No. 8737	2.88	.0048	.0054	----------	----------
2	Clarksville, No. 195	2.18	.0047	.0054	----------	----------
2	Manor, No. 298	1.81	.0044	.0054	----------	----------
5	Cecil, No. 6977	1.34	.0037	.0043	----------	----------
6	----do	1.34	.0041	.0046	740	550
1	Columbiana, No. 9804	.81	[1] .0053	.0047	550	400
7	Cecil, No. 6977 [2]	1.34	.0070	.0072	850	850

[1] Values a little too high (see footnote 1, experiment 1, table 1).
[2] Wheat was grown instead of millet.

It was necessary to ascertain whether the toxicity of sodium selenate is affected by variations in the available phosphate, since the phosphate supply presumably varied somewhat in the different sand-soil series. As mentioned previously, phosphate applications were varied according to the capacities of the different soils for phosphate fixation, and these capacities were merely estimated. The effect of different quantities of monocalcium phosphate on selenate toxicity in quartz sand is shown in experiments 3 and 4 in table 1. In experiment 3 the quantities of selenium required to reduce the yield one-half were 0.006, 0.0043, and 0.0044 g with 0.10, 0.20, and 0.30 g of P_2O_5, respectively; in experiment 4 the half-toxicity values were 0.0038 and 0.0046 g of selenium with 0.225 and 0.45 g of P_2O_5, respectively. These differences are hardly significant. Apparently, then, moderate quantities of available phosphate in excess of the plant's requirements have little or no influence on the toxicity of sodium selenate.

That sulphate ions diminish the toxicity of sodium selenate has been demonstrated by Hurd-Karrer (*8*). The degree of this influence, in terms of the half-toxicity value, is shown in part of experiment 1 and in experiment 6, table 1. In experiment 1 superphosphate was compared with monocalcium phosphate in the Columbiana sand-soil mixture. In the monocalcium phosphate series of this experiment the quantity of sulphate (SO_4) per pot was 0.24 g, whereas in the superphosphate series there must have been roughly 1 g of soluble SO_4 per pot. This increase in sulphate is doubtless responsible for raising the half-toxicity value from 0.0053 to 0.0080 g of selenium per pot. In experiment 6 the effects of 0.024 and 0.24 g of SO_4 per pot

were compared in quartz sand and in Marshall and Cecil soil-sand mixtures. With 0.024 g of SO_4 the half-toxicity values were 0.0025 g of selenium for all three mediums, whereas with 0.24 g of SO_4 the half-toxicity values were 0.0046, 0.0043, and 0.0041 g of selenium.

The toxicity values given in table 2 apparently are fairly accurate. The agreement of determinations duplicated in experiments 5 and 6 indicates that one-half a milligram in the toxicity values is sufficient allowance for errors and variations in the actual conduct of the experiments. Possible errors inherent in the method of experimentation may be somewhat larger than those involved in carrying out the experiments. The addition of soil undoubtedly renders the sand a better medium for growth, as shown by the comparative yields in sand and in sand-soil mixtures without selenium (table 1). That this improvement in conditions for growth does not increase, but rather decreases, the selenate needed to produce one-half injury is possibly due to the fact that it increases the absorptive capacity of the plant. At all events, plants in the sand-soil mixtures contain a higher concentration of selenium in the parts above ground than plants in pure sand, as shown in the last two columns of table 2. The results of the wheat experiment (last line of table 2, and experiment 7, table 1) confirm this idea. In this experiment the yield without selenate is about the same in pure sand as in the soil-sand mixture, and the concentration of selenium in the plant is practically the same for the two mediums.

The preceding experiments give no indication that soil colloids form a selenate compound unavailable to plants. But it is possible that soil colloids do have an effect on selenate which is too slight to be measured in experiments with only 1 percent of colloidal material. It is also possible that larger quantities of soil colloids might affect the plant's absorption of selenate in some way other than through the formation of an insoluble compound. Experiments were therefore conducted to measure the toxicity of sodium selenate in different whole soils.

EXPERIMENTS CONDUCTED WITH WHOLE SOILS

The experiments with whole soils were similar to those with the sand-soil mixtures just described. Each experiment included a series with pure quartz sand to serve as a standard for comparing the results of experiments conducted at different times. Eight soils received precipitated calcium carbonate as follows: The Columbiana and Nacogdoches soils, 1 g per pot; the Marshall, Cecil, Chester, and Muskingum soils, 2 g per pot; and the Manor and Keyport soils, 3 g per pot. The fertilizer applied to the soil consisted of 1.40 g of KNO_3, 0.33 g of $(NH_4)_2SO_4$, 0.136 g of NH_4Cl, and different quantities of monocalcium phosphate, depending on the estimated needs of the soil. This fertilizer is somewhat different from that applied to the sand-soil mixtures, but the quantity of sulphate applied is the same. Sodium selenate was added in solution along with the potassium and ammonium salts. The fertilizer applied to the quartz sand series was the same as in the work with sand-soil mixtures. Water added, and kept constant by weighing, was sufficient to give a uniform water content from top to bottom of the pot. The quantity was about 60 percent of the water-holding capacity of the soil and ranged in different soils from 715 to 1,250 cc per pot.

Most of the experiments were conducted to determine the quantities of selenate required to reduce the yield one-half in different soils. But experiments were also conducted to find to what extent the half-toxicity values were affected by the concentration of sulphate, by the quantity of phosphate, and by the manner in which the selenate was applied. The detailed results of the different experiments are given in table 3.

TABLE 3.—*Effect of different whole soils on the toxicity of sodium selenate to plant growth*

[Millet grown except as otherwise indicated]

EXPERIMENT 9 (JUNE 20 TO JULY 8)

Laboratory	Kind of soil	Depth	P_2O_5 applied per pot	Sodium selenate added per pot, expressed as Se	Air-dry yield of plants per pot				Selenium in air-dry plants	Half toxicity value Se required per pot to reduce yield one-half
					Series A	Series B	Series C	Average		
		Inches	*Grams*	*Gram*	*Grams*	*Grams*	*Grams*	*Grams*	*P. p. m.*	*Gram*
	Quartz sand	-------	0.15	0.000	2.10	2.54	2.19	2.28	0	.0043
				.005	.92	1.04	.89	.95	590	
				.010	.34	.37	.33	.35	-------	
6977	Cecil sandy clay loam	0–6	4.00	.000	4.88	4.94	4.54	4.79	-------	.0069
				.005	3.05	3.29	2.91	3.08	-------	
				.010	1.36	1.33	1.29	1.33	-------	
8736	Marshall silt loam	0–13	.40	.000	6.94	6.42	5.76	6.37	-------	.0050
				.005	3.26	3.24	3.00	3.17	-------	
				.010	1.02	.90	1.08	1.00	-------	

EXPERIMENT 10 (OCT. 11 TO NOV. 15), 0.24 G. SO_4 ADDED PER POT

	Quartz sand	-------	0.20	0.000	1.76	1.45	1.63	1.61	0	0.0046
				.005	.54	.84	.80	.73	550	
6977	Cecil sandy clay loam	0–6	4.00	.000	2.53	2.44	-------	2.49	0	.0054
				.005	1.10	1.57	-------	1.34	570	
8736	Marshall silt loam	0–13	.53	.000	3.38	3.00	-------	3.19	0	.0071
				.005	2.16	2.02	-------	2.09	560	

EXPERIMENT 10 (OCT. 11 to NOV. 15) NO SULPHATE ADDED TO SOILS; 0.024 G SO_4 PER POT ADDED TO SAND

	Quartz sand	-------	0.20	0.000	1.40	1.32	1.27	1.33	-------	0.0025
				.005	.01	.01	.04	.02	-------	
6977	Cecil sandy clay loam	0–6	4.00	.000	2.72	2.24	-------	2.48	-------	.0028
				.005	.30	.22	-------	.26	-------	
8736	Marshall silt loam	0–13	.53	.000	3.34	3.68	-------	3.51	-------	.0025
				.005	.03	.02	-------	.03	-------	

EXPERIMENT 11 (APR. 1 TO MAY 1) SELENATE ADDED AT TOP OF POT; LOW PHOSPHATE

	Quartz sand	-------	0.225	0.000	3.47	1.65	3.59	2.90	-------	0.0038
				.005	1.00	.48	1.50	.99	400	
				.010	.37	.50	.49	.45	-------	
6977	Cecil sandy clay loam	0–6	2.00	.000	5.28	5.78	5.64	5.57	-------	.0064
				.005	3.70	3.08	3.55	3.44	240	
				.010	1.09	1.07	-------	1.08	-------	
8736	Marshall silt loam	0–13	.20	.000	7.65	8.08	6.68	7.47	-------	.0041
				.005	2.92	2.90	2.78	2.87	400	
				.010	.87	.97	-------	.92	760	

TABLE 3.—*Effect of different whole soils on the toxicity of sodium selenate to plant growth*—Continued

EXPERIMENT 11 (APR. 1 TO MAY 1) SELENATE MIXED WITH SOIL; LOW PHOSPHATE

Laboratory	Kind of soil	Depth	P_2O_5 applied per pot	Sodium selenate added per pot, expressed as Se	Air-dry yield of plants per pot				Selenium in air-dry plants	Half toxicity value Se required per pot to reduce yield one-half
					Series A	Series B	Series C	Average		
		Inches	*Grams*	*Gram*	*Grams*	*Grams*	*Grams*	*Grams*	*P. p. m.*	*Gram*
6977	Cecil sandy clay loam	0–6	2.00	0.000	5.38	6.76	-------	6.07	-------	0.0079
				.005	4.28	5.06	4.16	4.50	240	
				.010	1.66	2.28	-------	1.97	530	
8736	Marshall silt loam	0–13	.20	.000	5.42	4.42	4.90	4.91	-------	.0072
				.005	3.52	3.33	3.00	3.28	220	
				.010	1.36	1.83	-------	1.60	440	

EXPERIMENT 11 (APR. 1 TO MAY 1) SELENATE ADDED AT TOP OF POT; HIGH PHOSPHATE

6977	Cecil sandy clay loam	0–6	4.00	0.000	5.20	6.85	5.90	5.98	-------	0.0066
				.005	3.40	3.78	4.22	3.80	180	
				.010	1.20	1.22	-------	1.21	450	
8736	Marshall silt loam	0–13	.40	.000	7.53	6.17	8.01	7.24	-------	.0048
				.005	3.43	3.77	3.21	3.48	380	
				.010	1.20	1.16	-------	1.18	590	

EXPERIMENT 12 (AUG. 29 TO SEPT. 26)

	Quartz sand	-------	0.15	0.000	2.58	2.51	2.88	2.66	0	0.0037
				.005	.83	.90	.94	.89	460	
				.010	.31	.38	.48	.39	1,100	
	Keyport clay loam	-------	2.70	.000	6.43	6.14	5.79	6.12	0	.0073
				.005	-------	3.59	4.13	3.86	220	
				.010	2.46	1.90	2.05	2.14	530	

EXPERIMENT 13 (FEB. 27 TO APR. 3)

	Quartz sand	-------	0.20	0.000	2.22	2.24	1.82	2.09	0	0.0041
				.005	.96	.76	.76	.83	640	
				.010	.32	.28	.39	.33	1,500	
B1086	Spearfish silt loam	0–12	.40	.000	5.77	6.44	4.62	5.61	0	.0072
				.005	3.26	3.88	3.72	3.62	260	
				.010	1.81	1.76	1.70	1.76	630	
	Clarion loam	-------	.40	.000	6.10	5.58	4.45	5.38	0	.0061
				.005	2.79	3.48	2.98	3.08	260	
				.010	.88	1.80	1.11	1.26	340	
298	Manor loam	7–20	2.00	.000	4.88	4.80	5.40	5.03	0	.0074
				.005	3.44	3.48	3.28	3.40	250	
				.010	1.81	1.46	1.41	1.56	430	
9804	Columbiana clay	0–10	3.00	.000	3.03	2.90	3.02	2.98	0	
				.005	3.04	2.82	2.91	2.92	110	
				.010	2.82	2.28	2.52	2.54	130	

EXPERIMENT 14 (MAY 24 TO JUNE 18)

	Quartz sand	-------	0.20	0.000	4.36	3.94	3.78	4.03	0	0.0042
				.005	1.95	1.38	1.53	1.62	620	
				.010	.62	.60	.64	.62	970	
300	Chester loam	0–8	2.00	.000	4.46	4.83	4.11	4.47	0	.0071
				.005	2.82	2.88	2.74	2.81	610	
				.010	1.41	1.42	1.71	1.51	-------	
6718 6719	Vernon fine sandy loam	0–10	.40	.000	4.77	4.76	4.60	4.71	0	.0069
				.005	3.13	2.95	2.60	2.89	340	
				.010	1.35	1.12	1.09	1.19	840	
6842	Colby silty clay loam	2–10	.20	.000	5.97	7.57	7.88	7.14	1	.0060
				.005	3.92	4.10	4.08	4.03	350	
				.010	1.56	1.58	2.00	1.71	570	
9804	Columbiana clay	0–10	5.62	.000	4.88	4.88	-------	4.88	0	.0164
				.020	2.02	1.88	-------	1.95	540	
				.030	.16	.42	-------	.29	780	

TABLE 3.—*Effect of different whole soils on the toxicity of sodium selenate to plant growth*—Continued

EXPERIMENT 15 (SEPT. 1 TO SEPT. 24)

Laboratory	Kind of soil	Depth	P_2O_5 applied per pot	Sodium selenate added per pot, expressed as Se	Air-dry yield of plants per pot				Selenium in air-dry plants	Half toxicity value Se required per pot to reduce yield one-half
					Series A	Series B	Series C	Average		
		Inches	*Grams*	*Gram*	*Grams*	*Grams*	*Grams*	*Grams*	*P. p. m.*	*Gram*
	Quartz sand		0.20	0.000	3.10	3.29	3.09	3.16		
				.005	1.10	.96	1.01	1.02	750	0.0037
				.010	.33	.30	.40	.34	1,100	
B407	Muskingum silt loam	0–7	.27	.000	5.70	5.71	6.09	5.83	0	
				.005	3.86	3.42	4.29	3.86	230	.0074
				.010	2.14	1.73	1.76	1.88	660	
				.000	3.18	3.60	3.44	3.41	1	
6679	Kirvin fine sandy loam	12–24	2.70	.0075	2.02	2.30	2.13	2.15	540	.0101
				.015	.79	.97	.88	.88	1,100	
				.000	3.70	3.78	3.44	3.64	1	
6678	do	0–12	.70	.005	1.98	2.28	1.75	2.00	180	.0057
				.010	.59	.52	.41	.51	850	

EXPERIMENT 16 (OCT. 12 TO NOV. 13)

	Quartz sand		0.20	0.000	2.00	2.31	2.63	2.31		
				.005	1.16	1.22	1.26	1.21	630	0.0053
				.010	.43	.36	.32	.37	810	
				.000	4.15	2.96	2.44	3.18	0	
9475	Nacogdoches fine sandy loam	0–8	2.70	.0075	.78	.85	.74	.79	510	.0050
				.015	.10	.25	.30	.22	910	
				.000	4.36	4.66	4.07	4.36		
300	Chester loam	0–8	2.00	.005	3.19	2.72	3.17	3.03	230	0.0080
				.010	1.50	1.75	1.66	1.64	200	

EXPERIMENT 17 (NOV. 27 TO JAN. 14) WHEAT GROWN INSTEAD OF MILLET

	Quartz sand		0.15	0.000	2.92	3.32	3.24	3.16	1	
				.010	.82	.96	1.08	.95	1,700	0.0072
				.020	.27	.28	.28	.28	2,500	
6977	Cecil sandy clay loam	0–6	4.00	.010	2.52	2.67		2.60	750	
				.020	.88	.84		.86	1,600	.0152
				.000	3.14	3.02		3.08	0	
8736	Marshall silt loam	0–13	.40	.010	1.08	1.16		1.12	1,200	0.078
				.020	.20	.15		.18	2,000	

The extent to which the sulphate application affects the toxicity of sodium selenate in the Marshall and Cecil soils is shown in table 3, experiment 10, in which the plants were grown with no sulphate added to the soil and with the addition of 0.24 g of SO_4 per pot. The addition of 0.24 g of SO_4 per pot changed the toxicity value in the Marshall soil from 0.0025 to 0.0071 g of selenium, and in the Cecil soil from 0.0028 to 0.0054 g of selenium. These results with whole soils are similar to those obtained with sand-soil mixtures, and indicate that the influence of the sulphate is about the same for the two mediums.

The effect of phosphate on selenate toxicity is shown in experiment 11, table 3. Doubling the quantity of phosphate applied raised toxicity values in the different soils as follows: From 0.0064 to 0.0066 g of selenium per pot in the Cecil soil, and from 0.0041 to 0.0048 in the Marshall soil. The yields of the pots to which no selenium was added show that the double phosphate application supplied at least twice as much phosphate as was needed. It may be concluded, therefore,

that the toxicity values of the whole soils are not appreciably affected by variations in the phosphate fertilization, up to at least double the requirements of the soil and plant.

In the experiments with whole soils as well as in the preceding experiments with sand-soil mixtures, the sodium selenate and the soluble fertilizer salts were added to the dry soil, dissolved in the total amount of water needed to bring the soil to the optimum water content. Certainly in the case of sand cultures this seemed the best method of applying the selenate, since it gave a uniform distribution of selenate throughout the sand at the beginning of the experiment. It was a question, however, what the distribution of selenate would be at the end of the experiment. It was also questionable whether this method of application would give as fair a comparasion of toxicity values in different whole soils as mixing the selenate with the soil after adding most of the water.

The distribution of selenate in soil and in sand at the end of an experiment was studied by sampling the top, middle, and bottom thirds of soil in a pot and analyzing the three layers for selenium. Pots receiving the larger selenate application in experiment 7, table 1, and experiments 11 and 17, table 3, were subjected to this procedure. The results are given in table 4, together with the half-toxicity values of the soils and the selenium content of the plants above the ground at one-half injury.[3]

TABLE 4.—*Distribution of selenate in different parts of a pot at the end of an experiment*

[Pots receiving larger selenate application in experiment 7, table 1, and experiments 11 and 17, table 3]

Experiment No.	Sodium selenate applied per pot, as gram of selenium	Kind of soil	Crop	How selenate was applied	Selenium present in different layers of soil, as parts per million of soil			Half-toxicity value (selenium required to reduce yield one-half)	Selenium in crop at one-half injury
					Top third	Middle third	Bottom third		
	Gram				*P. p. m.*	*P. p. m.*	*P. p. m.*	*Gram*	*P. p. m.*
7	0.02	Quartz sand	Wheat	At top	2.0	3.5	5.0	0.0072	1,225
7	.02	Sand and Cecil soil	do	do	3.0	3.5	3.5	.0070	1,210
17	.02	Whole Cecil soil	do	do	6.0	3.5	1.0	.0152	1,175
17	.02	Whole Marshall soil	do	do	5.0	3.5	2.0	.0078	900
11	.01	Whole Cecil soil	Millet	do	5.0	3.5	.6	.0064	310
11	.01	do	do	Mixed	2.5	3.0	3.5	.0079	410
11	.01	Whole Marshall soil	do	At top	3.5	3.0	3.0	.0041	330
11	.01	do	do	Mixed	1.0	3.5	5.0	.0072	320

Evidently when the sodium selenate is added at the top of the pot it works downward somewhat in the quartz sand, becomes almost uniformly distributed in the sand-soil mixture, and remains more concentrated in the upper layers in whole soils. On the other hand, when the selenate is mixed uniformly with the whole soil, it tends to become more concentrated in the lower layers during the experiment. In the Marshall soil the selenate evidently moves more freely than in the Cecil. Differences in distribution of the selenate were not due to differences in distribution of moisture, since the water content of

[3] Determination of selenium in the soil samples were made by K. T. Williams.

the three layers of soil was found to be the same within a few tenths of a percent.

The results of the millet experiment (table 4) show that the toxicity of the selenate varies appreciably with its distribution in the soil. Apparently the toxicity is less, that is, the toxicity value is greater, when the selenate accumulates in the lower part of the pot. And since this downward movement is freer in some soils than in others, it should make for inaccuracy in comparing the toxicities of selenate in different soils. However, if it is permissible to generalize from the results of the two soils, the Cecil and the Marshall, it seems that the downward movement is less in soils in which the toxicity is less, that is, the toxicity value is greater. Hence, if a correction were made for inequalities of distribution in different soils, it would involve increasing somewhat the larger toxicity values. One method of application does not seem more accurate than the other for comparing the effects of different soils on selenate toxicity. Therefore, the application in solution was continued, this being the less laborious method.

Experiments to determine to what extent the toxicity of sodium selenate varies in different soils are reported in detail in table 3. The toxicity values for the 14 soils tested and for quartz sand in the same experiments are summarized in table 5. By subtracting the toxicity values for sand from the toxicity values for soils, figures were obtained for the effects of the total colloidal fractions on selenate toxicity. These figures, given in next to the last column, are based on the assumption that the noncolloidal soil material would have the same toxicity value as quartz sand. The specific effects of the different soil colloids, based on the same assumption, are given in the last column. These were obtained by dividing the figure representing the effect of the whole colloid fraction by the percentage of colloid in the soil. All values are given as milligrams of selenium per pot of soil or sand.

TABLE 5.—*Toxicity of sodium selenate in different whole soils and calculated effects of the colloids*

Experiment No.	Kind of soil	Quantity of colloid in soil	Molecular ratio of SiO_2 to Al_2O_3+Fe_2O_3 in colloidal material	Half-toxicity value [1] for the soil	Half-toxicity value [1] for quartz sand in same experiment	Difference between half-toxicity [1] values for soils and for sand	Calculated half-toxicity [1] value for 1 percent of soil colloid per pot
		Percent		*Mg*	*Mg*	*Mg*	*Mg*
14	Colby, No. 6842	30.2	3.41	6.0	4.2	1.8	0.06
9	Marshall, No. 8736	32.4	2.91	5.0	4.3	.7	.02
10	do	32.4	2.91	7.1	4.6	2.5	.08
11	do	32.4	2.91	4.1	3.8	.3	.01
13	Spearfish, No. B1086	23.7	2.84	7.2	4.1	3.1	.13
13	Clarion			6.1	4.1	2.0	
14	Vernon, Nos. 6718–19	8.5	2.50	6.9	4.2	2.7	.31
15	Muskingum, No. B407	19.9	2.26	7.4	3.7	3.7	.19
15	Kirvin, No. 6678	5.6	2.02	5.7	3.7	2.0	.36
12	Keyport			7.3	3.7	3.6	
13	Manor, No. 298	18.1	1.81	7.4	4.1	3.3	.18
15	Kirvin, No. 6679	59.4	1.74	10.1	3.7	6.4	.11
14	Chester, No. 300	14.3	1.54	7.1	4.2	2.9	.20
16	do			8.0	5.3	2.7	.20
9	Cecil, No. 6977	17.3	1.34	6.9	4.3	2.6	.15
10	do			5.4	4.6	.8	.05
11	do			6.4	3.8	2.6	.15
16	Nacogdoches, No. 9475	16.1	1.07	5.0	5.3	−.3	
14	Columbiana, No. 9804	48.0	.81	16.4	4.2	12.2	.25

[1] Se required per pot to reduce yield one-half. See text for full explanation.

The accuracy of the experimental figures is roughly indicated by the small differences in the toxicity values for quartz sand in different experiments. Apparently, the experimental figures in columns 5, and 6 are for the most part accurate within a milligram although certain determinations, such as those for the Marshall and Cecil soils in experiment 10, are probably less exact.

The significant features of the data in table 5 are: The general uniformity in the toxicity values for different soils; the very low values for the calculated specific effects of the different soil colloids; the lack of relation between the specific effects of the colloids and the silica-sesquioxide ratios of the colloids; and the fact that all soils except the Nacogdoches have higher toxicity values than quartz sand.

These results might be explained on the ground that a soil colloid has a slight capacity for forming a selenate compound which is insoluble and unavailable to the plant and that this capacity is not related to the silica-sesquioxide ratio. It is believed, however, that this is not the true explanation. It seems rather improbable that if the soil colloid has any capacity for forming an insoluble selenate it should be so small and should bear no relation to the silica-sesquioxide ratio. Also, it seems significant that soils containing widely different amounts of different kinds of colloid should have toxicity values so nearly alike.

Another explanation of the results is suggested by data, shown in table 4, on the movement of selenate towards the bottom of the pot during an experiment. The different distributions of sodium selenate in sand and soils evidently do not, in themselves account for the different toxicities found in these mediums. But these data on downward movement may be taken as indicating that mobility of the selenate in sand is greater than in soil and that mobility in the Marshall soil is greater than in the Cecil. Differences in mobilities might account for the different toxicities of selenate in the various mediums, for it is quite conceivable that toxicity of the selenate increases with its mobility in the medium of growth. A free movement of selenate into the area immediately contiguous to the roots, for instance, should promote absorption of selenate by the plant, since absorbed selenate would be rapidly renewed from other areas. The less mobility of selenate in soil than in sand may be due to differences in structure or it may be due to an attraction between the colloid and the selenate which is weaker than that existing where an insoluble compound is formed. In either case it would not be expected to parallel closely the chemical composition of the colloid.

The preceding explanation of the experimental results on the basis of mobility of the selenate is quite hypothetical, for we have no data regarding the movement of selenate into the area where absorption takes place and only presume that it is freer where the downward movement of the salt in the pot is freer. The explanation would account, however, for the less toxicity (higher half-toxicity values) of selenate in soils than in sand, the comparatively little variation in the toxicity values of different soils, and the lack of relation between the chemical composition of the colloid and its effect on the selenate.

The experimental results with whole soils, no matter how they are explained, show quite clearly that the toxicity of sodium selenate does not vary markedly in different soils. Both series of experiments, those with whole soils and the preceding ones with sand-soil mixtures,

indicate that if soil colloids have any specific effect on the selenate ion it is very small and does not parallel the silica-sexquioxide ratio.

SELENIUM CONTENT OF THE PLANTS

Many of the plants grown in the preceding experiments were analyzed for selenium, since it seemed possible that the selenium in the plant might furnish a better criterion of the soil's effect on selenate toxicity than the reduction in yield. Only the part of the plant above ground was analyzed. The roots doubtless contained some selenium, but Hurd-Karrer (*10*) has shown that practically all the selenate absorbed by certain plants is located in the part above ground.

Analyses were made in many cases of the crops grown with both 0.005 and 0.010 g of selenium per pot. Figure 2 summarizes the

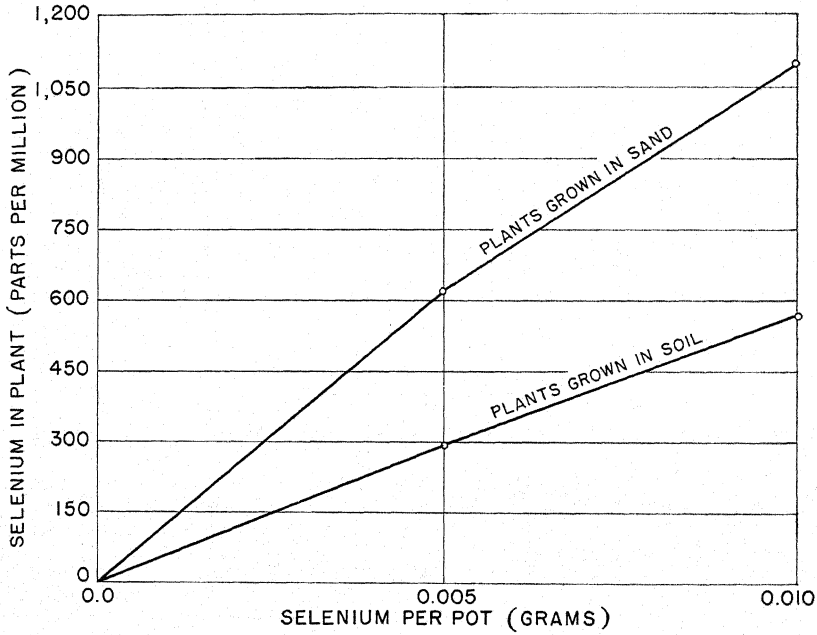


FIGURE 2.—Effect of increasing quantities of sodium selenate on the selenium content of millet.

results. The curve for quartz sand is an average of the results obtained with quartz sand in 5 experiments, Nos. 12 to 16, and the curve for soils is an average of the results obtained with 13 different soils in these experiments and in experiment 11. One of the results obtained with quartz sand and three of the results with soils gave curves differing widely from the average curves shown in figure 2, but for the most part individual curves were close to the average in form. The curves show that the selenium content of the plant above ground tends in general to increase in direct proportion to the quantity of sodium selenate applied. This holds for millet up to the point where the selenate is sufficient to inhibit yield appreciably, but may not hold for plants much more tolerant of selenate. Because of this direct proportionality between selenate applied and selenium in the plant, it was possible to calculate the selenium content of plants for selenate applications producing half injury.

If the selenium content of plants grown in quartz sand at different times is considered first, the data on plants grown in soil will be better understood. Tables 1 and 3 contain 11 different determinations of the quantity of sodium selenate reducing the yield of millet one-half in quartz sand and 9 determinations of the selenium content of the crops grown with an application of 0.005 g of selenium to the quartz sand. These data are brought together in table 6, together with figures calculated for the selenium content of the plants at one-half injury. The quartz sand series of experiments Nos. 9, 10, and 11 are not included in the table, since they are the same sand series that appear in experiments 5, 6, and 4, respectively. These experiments were run in pairs.

TABLE 6.—*Toxicity of sodium selenate in quartz sand as related to selenium in millet plants, determined in different experiments*

Experiment No.	Half-toxicity value Se required per pot to reduce yield one-half	Se in plants grown with 0.005 g of Se per pot	Calculated Se in plants at one-half injury point	Experiment No.	Half-toxicity value Se required per pot to reduce yield one-half	Se in plants grown with 0.005 g of Se per pot	Calculated Se in plants at one-half injury point
	Gram	*P. p. m.*	*P. p. m.*		*Gram*	*P. p. m.*	*P. p. m.*
1	0.0047	400	380	15	0.0037	750	550
2	.0054	----------	----------	16	.0053	630	640
3	.0043	----------	----------				
4	.0038	400	300	Average	.0044	560	480
5	.0043	590	510				
6	.0046	550	510	Standard deviation	.00056	111	105
12	.0037	460	340	Coefficient of variability	*Percent* 13	*Percent* 20	*Percent* 22
13	.0041	640	520				
14	.0042	620	530				

It will be seen that the data obtained in different experiments vary, the half-toxicity figures being more constant than figures representing the selenium content of the plants. Most of the differences in half-toxicity values obtained in different experiments are strictly experimental error, since they are of about the same magnitude as the probable error of one determination calculated from the crop weights of triplicated pots in a single experiment. For instance, the standard deviation of toxicity values in column 2, table 6, is 0.00056 g, and the probable errors of the last three values in this column, calculated from the probable errors of the average crop weights in an experiment, are, respectively, 0.0006, 0.0002, and 0.0005 g.

Since the variation in half-toxicity values determined at different times of the year is not appreciably greater than the variation expected of duplicate determinations conducted at the same time, it is obvious that the toxicity of sodium selenate is not appreciably affected by differences in light, temperature, etc., obtaining in an ordinary glass house at different times of the year. This conclusion is rather surprising, for the intensity and duration of sunlight differed widely between some of the experiments. In two experiments where growth conditions differed so much that the check pots receiving no selenate yielded crops weighing 4.03 and 2.09 g, the selenate applications producing half injury were, respectively, 0.0042 and 0.0041 g of selenium per pot.

The coefficients of variability show that the parts per million of selenium in plants grown with 0.005 g of selenium and the parts per

million of selenium in the plants at half injury are more variable than the selenium applications producing half injury. Analytical error could not account for this, so far as known, unless it be that selenium is lost from the plant on air drying. It would seem to follow, then, that the toxicity of absorbed selenate varies somewhat within the plant and that more selenate is taken up when conditions are such that it is less toxic. This conclusion, however, is far from certain. It may be that there was sufficient selenium in the roots, which were not analyzed, to equalize discrepancies in the selenium content of the plants above ground. It is also possible that the selenium content of the plant varies with the stage of maturity and that the plants were harvested at somewhat different stages of maturity. The stage of development at which the plants were cut (at jointing) was only approximately the same. In fact, it is somewhat of a question just what constitutes comparable stages of development in short- and long-day periods if the development of the plant varies with length of day. Until the factors responsible for the variable selenium content of the plants are established, the half-toxicity determination may be considered a better index of the soils' effect on selenate toxicity.

The selenium content of millet grown in different soils receiving 0.005 g of selenium per pot is shown in table 7. The table also shows the selenium content of plants grown in quartz sand in the same experiment, and the selenium application reducing the yield one-half in the soil.

TABLE 7.—*Se content of millet grown in soil and in quartz sand with sodium selenate applied at rate of 0.005 g Se per pot*

Experiment No.	Kind of soil	Half-toxicity value for soil (Se required per pot to reduce yield one-half)	Se content of plants grown—		Se content of soil-grown plants as percentage of Se content of quartz sand-grown plants
			In soil	In quartz sand in same experiment	
		Mg	*P. p. m.*	*P. p. m.*	*Percent*
14	Colby, No. 6842	6.0	350	620	56
10	Marchall, No. 8736	7.1	560	550	102
11	do	4.1	400	400	100
13	Spearfish, No. B1086	7.2	260	640	41
13	Clarion	6.1	260	640	41
14	Vernon, Nos. 6718–19	6.9	340	620	55
15	Muskingum, No. B407	7.4	230	750	31
15	Kirvin, No. 6678	5.7	180	750	24
12	Keyport	7.3	220	460	48
13	Manor, No. 298	7.4	250	640	39
15	Kirvin, No. 6679	10.1	360	750	48
14	Chester, No. 300	7.1	610	620	98
16	do	8.0	230	630	37
10	Cecil, No. 6977	5.4	570	550	104
11	do	6.4	240	400	60
16	Nacogdoches, No. 9475	5.0	340	630	54
14	Columbiana, No. 9804	16.4	130	620	21

Practically all the plants grown in soil have a lower selenium content than the corresponding plants grown in sand. The data indicate quite clearly, then, that sodium selenate is somewhat less toxic in nearly all soils than in quartz sand. The half-toxicity figures previously discussed led to the same conclusions, so in a general way the analytical data may be taken as supporting the growth data.

Although in general there is this agreement between toxicity values and selenium content, the variations in selenium content of plants from different soils do not follow the variations in toxicity values at all well, the correlation coefficient between the two sets of figures being −0.37. This was to be expected from the quartz-sand data in table 6, which showed that toxicity values obtained in different experiments were comparatively constant but selenium content of the plants varied. It seemed as though variations induced by growing the plants in six different experiments might be eliminated by expressing the selenium content of the soil-grown plants as percentages of the selenium content of the plants grown in quartz sand in the same experiment. The figures obtained in this way are given in the last column of table 7. These agree with the toxicity values only slightly better, the correlation coefficient being −0.44.

Neither the selenium content of the soil-grown plants (column 4, table 7) nor the relative selenium content (last column, table 7) shows an appreciable correspondence with the silica-sesquioxide ratios of the soil colloids or with the specific effects of the colloids given in table 5. The growth data in table 5, previously discussed, likewise failed to show an appreciable correspondence with the silica-sesquioxide ratios of the colloids.

In a general fashion, the selenium content of the plants supports the conclusions indicated by the half-toxicity values based on growth, but it does not seem to give any added information. The half-toxicity values, in being more closely reproducible, seem the more accurate criterion of selenate toxicity. Doubtless, with further investigation of the factors influencing the selenium content of the plant, these figures would be more valuable.

TESTS WITH PLANTS OTHER THAN MILLET

The data that have been considered were all obtained with millet. Had other plants been used, the figures would no doubt have been different, but the general results the same. Millet is more sensitive to selenate than many plants, but not exceptionally so. In a test with mustard with quartz sand (table 1, experiment 8), half injury was produced by 0.0051 g of selenium per pot from sodium selenate—a figure near the average for millet. Marquis wheat grown for 48 days (table 1, experiment 7), seems to be considerably more tolerant of selenate than millet. The half-toxicity figures obtained in quartz sand, in a Cecil soil-sand mixture, and in the Marshall soil (table 4) range from 0.0070 to 0.0078 g of selenium per pot (or about one and one-half times the figure for millet. The figure for wheat in the whole Cecil soil is about two-and-a-half times that for millet). The selenium content of the wheat plants above ground at one-half inquiry was about two times the average for millet in quartz sand.

GENERAL CONCLUSIONS

The foregoing experiments indicate that the toxicity of sodium selenate should vary little in different soils. Under comparable conditions of moisture and fertilization, most soils should tolerate about one and one-half times as much selenate as quartz sand, and an exceptional soil, such as the lateritic Columbiana, might stand three to four times as much as the sand. If the data obtained in 1-gallon pots

are calculated to an acre 6 inches of soil, figures are obtained which may be incorrect by several hundred percent but are easy to comprehend. The application of 0.0044 g of selenium per pot, which reduced the yield of millet one-half in a pot of quartz sand, is equivalent to a little less than 1 part of selenium per million parts of soil (0.88 p. p. m.), and the application producing half injury in the average soil is a little less than 1½ parts of selenium per million, or about 3 pounds per acre. As a matter of fact, this figure of 1½ p. p. m. or 3 pounds per acre, is not fantastic. Byers (*3*) reports selenium absorption by certain plants from soils containing such quantities of selenium and an apparent effect on the flora. Moreover, boron applications of this general magnitude have been found distinctly injurious on some soils. It should be understood that selenate is only one of the forms in which selenium occurs in soils (*16*).

Apparently the toxicity of sodium selenate may vary more under different conditions in one and the same type of soil than under comparable conditions in different types of soil. With a low supply of sulphate in the soil, for instance, half as much selenate as the quantities mentioned above may produce notable injury. Variations in the vertical distribution of the selenate should also affect the toxicity in the field. The effect of variation in the water supply was not studied, but this may influence toxicity.

The soil colloids seem to have only a slight influence on the toxicity of sodium selenate. Experiments in sand-soil mixtures containing 1 percent of colloid gave no indication that the soil colloid reduces selenate toxicity. But in most whole soils the selenate was less toxic than in quartz sand, and this difference in toxicity presumably should be attributed to the colloids in the soils. It does not seem that the small reduction in selenate toxicity in whole soils is due to a specific reaction between colloid and selenate, resulting in a compound unavailable to the plant. It seems rather that the reduced toxicity is due to a more general effect of the colloids on distribution or movement of the selenate in the soil medium.

SUMMARY

This investigation deals with the effects of soil colloids on the toxicity of sodium selenate to millet, as determined by vegetative experiments in pots. The measure of toxicity employed is the quantity of selenate required to reduce yield one-half.

Experiments in quartz sand, in soil, and in sand-soil mixtures show that the toxicity of sodium selenate is not appreciably affected by phosphate fertilization, but is affected by the vertical distribution of selenate in the pot and by sulphate fertilization. When the sulphate application is high, the selenate application needed to reduce yield one-half may be two to three times that needed when the sulphate supply is adequate only for maximum yield.

With a constant sulphate fertilization, sodium selenate is as toxic in sand-soil mixtures containing 1 percent of different soil colloids as in pure quartz sand, but is somewhat less toxic in whole soils. It does not seem that the small reduction in selenate toxicity in whole soils, as compared with quartz sand, is due to a specific reaction between colloid and selenate, resulting in a compound unavailable to the plant. The reduction in toxicity seems rather to be due to a

general effect of the colloids, possibly on movement of selenate in the soil medium. Differences between the toxicities of selenate in sand and in 14 soils bear no relation to the silica-sesquioxide ratios of the soil colloids.

The quantity of selenate required to reduce yield one-half is unaffected by the different growth conditions obtaining in an ordinary greenhouse at different times of the year. The selenium content of the plants at half injury is more variable than the half-toxicity values.

Marquis wheat grown for 48 days requires one and one-half times as much selenate to reduce yield one-half as millet grown to the jointing stage, and at half injury the part above ground contains about twice as much selenium.

The data obtained in 1-gallon pots indicate that about one and one-half parts per million of selenium as selenate, or 3 pounds per acre 6 inches, should be distinctly injurious to millet in most soils. This calculation from a pot to an acre is of course not reliable, but the figure is in harmony with field observations.

The preceding conclusions apply only to selenium in the form of sodium selenate. In work now in progress quite different results are being obtained with sodium selenite.

LITERATURE CITED

(1) BROWN, IRVIN C., and BYERS, HORACE G.
1935. THE CHEMICAL AND PHYSICAL PROPERTIES OF DRY-LAND SOILS AND OF THEIR COLLOIDS. U. S. Dept. Agr. Tech. Bull. 502, 56 pp.

(2) BYERS, HORACE G.
1935. SELENIUM OCCURRENCE IN CERTAIN SOILS IN THE UNITED STATES, WITH A DISCUSSION OF RELATED TOPICS. U. S. Dept. Agr. Tech. Bull. 482, 48 pp., illus.

(3) ———
1936. SELENIUM OCCURRENCE IN CERTAIN SOILS IN THE UNITED STATES, WITH A DISCUSSION OF RELATED TOPICS. SECOND REPORT. U. S. Dept. Agr. Tech. Bull. 530, 79 pp., illus.

(4) ——— ALEXANDER, LYLE T., and HOLMES, R. S.
1935. THE COMPOSITION AND CONSTITUTION OF THE COLLOIDS OF CERTAIN OF THE GREAT GROUPS OF SOILS. U. S. Dept. Agr. Tech. Bull. 484, 39 pp.

(5) GILE, PHILIP L.
1933. THE EFFECT OF DIFFERENT COLLOIDAL SOIL MATERIALS ON THE EFFICIENCY OF SUPERPHOSPHATE. U. S. Dept. Agr. Tech. Bull. 371, 50 pp., illus.

(6) ———
1936. THE EFFECT OF DIFFERENT COLLOIDAL SOIL MATERIALS ON THE TOXICITY OF CALCIUM ARSENATE TO MILLET. Jour. Agr. Research 52: 477–491, illus.

(7) GILE, P. L., MIDDLETON, H. E., ROBINSON, W. O., FRY, W. H., and ANDERSON, M. S.
1924. ESTIMATION OF THE COLLOIDAL MATERIAL IN SOILS BY ADSORPTION. U. S. Dept. Agr. Bull. 1193, 42 pp.

(8) HURD-KARRER, ANNIE M.
1934. SELENIUM INJURY TO WHEAT PLANTS AND ITS INHIBITION BY SULPHUR. Jour. Agr. Research 49: 343–357, illus.

(9) ———
1935. FACTORS AFFECTING THE ABSORPTION OF SELENIUM FROM SOILS BY PLANTS. Jour. Agr. Research 50: 413–427, illus.

(10) ———
1937. COMPARATIVE TOXICITY OF SELENATES AND SELENITES TO WHEAT. Amer. Jour. Bot. 24: 720–728, illus.

(11) MIDDLETON, H. E., SLATER, C. S., and BYERS, HORACE G.
1932. PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE SOILS FROM THE EROSION EXPERIMENT STATIONS. U. S. Dept. Agr. Tech. Bull. 316, 51 pp.

(12) ———
SLATER, C. S., and BYERS, H. G.
1934. THE PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE SOILS FROM THE EROSION EXPERIMENT STATIONS—SECOND REPORT. U. S. Dept. Agr. Tech. Bull. 430, 63 pp., illus.

(13) MITSCHERLICH, EILH. ALFRED.
1913. BODENKUNDE FÜR LAND- UND FORSTWIRTE. Aufl. 2, vollständig umgearb., 317 pp., illus. Berlin.

(14) ROBINSON, W. O., and HOLMES, R. S.
1924. THE CHEMICAL COMPOSITION OF SOIL COLLOIDS. U. S. Dept. Agr. Bull. 1311, 42 pp.

(15) SLATER, C. S., HOLMES, R. S., and BYERS, H. G.
1937. TRACE ELEMENTS IN THE SOILS FROM THE EROSION EXPERIMENT STATIONS, WITH SUPPLEMENTARY DATA ON OTHER SOILS. U. S. Dept. Agr. Tech. Bull. 552, 24 pp.

(16) WILLIAMS, K. T., and BYERS, H. G.
1936. SELENIUM COMPOUNDS IN SOILS. Indus. and Engin. Chem. 28: 912–914.

(17) ——— and LAKIN, H. W.
1935. DETERMINATION OF SELENIUM IN ORGANIC MATTER. Indus. and Engin. Chem., Analyt. Ed. 7: 409–410.

GROWTH SUBSTANCE AND THE DEVELOPMENT OF CROWN GALL[1]

By S. B. LOCKE, *research assistant in plant pathology*, A. J. RIKER, *professor of plant pathology*, and B. M. DUGGAR, *professor of botany and plant pathology, Wisconsin Agricultural Experiment Station.*[2]

INTRODUCTION

Advances made recently in the study of plant growth substances and their function in normal growth have raised the question of their possible role in atypical and pathological growth and specifically in the gall development induced by *Phytomonas tumefaciens* (Smith and Town.) Bergey et al.

A suggestion of their presence was first given by the marked epinasty of leaf petioles in plants inoculated with the crown gall organism. Other responses, including the initiation of adventitious roots, stimulation of cambial activity, inhibition of bud development, and delayed abscission of senescent leaves, strengthened this suggestion. The relation of these responses to growth substances in general are discussed by Boysen-Jensen (*3*).[3] Most of these phenomena have doubtless been frequently observed by those studying crown gall, but so far as the writers are aware only Němec (*25*) has interpreted any of them in relation to growth substances. Some of the literature providing a background for this problem is very briefly considered.

Epinasty of leaf petioles in tomato, resulting from the rapid elongation of cells on the upper side of the petioles, has been induced by beta-indole-acetic acid, and by numerous other compounds (*7*, *8*, *10*, *11*).

Initiation of adventitious roots in response to treatment with various growth substances has been frequently reported. Unsaturated hydrocarbon gases (*44*, *45*, *46*), pure auxins (*40*, cf. *43*), beta-indole-acetic acid (*39*), and certain other synthetic compounds (*47*) were demonstrated to stimulate the production of root initials. Initiation of roots in conjunction with crown gall was apparently observed early in the history of the disease (*33*) and was confirmed later (*5*, *25*). However, this is not to be confused with the hairy root condition caused by *Phytomonas rhizogenes* R. B. W. K. S.

Growth substances were found (*37*) to stimulate the cambium. Such stimulation was also obtained with beta-indole-acetic acid (*36*). Recently (*1*) cambial activity has been found to begin at the terminal buds and to progress basipetally following the movement of the growth substance. Stimulation of the cambium in the vicinity of crown gall tissue has also been noted (*26*, *32*).

Apical dominance and inhibition of axillary buds was at first attributed to a specific retarding substance (*34*, *35*) which was later considered to be identical with growth substance (*41*, *42*). Inhibition of axillary buds has been obtained with beta-indole-acetic, beta-indole-

[1] Received for publication November 12, 1937; issued July 1, 1938. This work was supported in part by the special research fund of the University of Wisconsin and by the International Cancer Research Foundation.

[2] The writers are indebted to Eugene Herrling for preparing the photographs.

[3] Italic numbers in parentheses refer to Literature Cited, p. 37.

propionic (*11*, *39*), and several other indole acids (*2*). The effect of crown gall upon bud development has been disputed. Smith (*29*, *30*, *31*) obtained leafy galls by inoculating internodes and axillary buds with crown gall bacteria, and explained it by stimulation of cells already possessing the potentiality of shoot formation. However, Levine (*18*, *19*, *20*) obtained opposing results and observed an inhibitive effect on the leaf-notch meristems of *Bryophyllum*. Němec (*25*) observed that inoculation of chicory root cuttings on the upper cut surface prevented the usual formation of shoots there. He concluded that growth substance produced by the bacteria was responsible for this effect.

Abscission of leaf petioles commonly follows closely after the deterioration or removal of the leaf blades which they support. Laibach (*13*) found that orchid pollen prevented the falling off of petioles, and he ascribed this effect to growth substance. LaRue (*17*) obtained inhibition of petiole abscission in *Coleus* with growth substances from leaves, pollen, and urine, and with beta-indole-acetic acid.

Growth substances have been found to stimulate the development of "callus," an effect which approaches the characteristic early consequence of inoculation with *Phytomonas tumefaciens*. Laibach and associates (*14*, *15*, *16*) stimulated callus development on stems of *Coleus*, and *Tradescantia* with extracts of pollen and of urine and also by applying a paste of beta-indole-acetic acid in lanolin to decapitated plants of *Vicia faba* Linn. Brown and Gardner (*6*) and Link, Wilcox, and Link (*22*) have also stimulated growth with substances isolated from crown gall cultures and applied to decapitated plants of *Phaseolus vulgaris* Linn. These authors produced similar proliferations with beta-indole-acetic acid. The histological developments produced by application of beta-indole-acetic acid resemble closely many of those associated with crown gall (*12*). Following Smith (*30*), Riker and Berge (*27*) list numerous other chemical agents capable of stimulating cell division locally and say, "The great diversity of materials reported to cause stimulation suggests that irritation or injury induced by them, which is apparently a common characteristic, is more important than the nature of the substance." Levine and Chargaff (*21*) recently tested several chemical fractions of crown gall bacteria and found the phosphatide fraction the most active in producing cell proliferations. It seems uncertain at present which, if any, of the chemicals listed belong to the group of growth substances under consideration here.

From these and related investigations it appeared desirable to examine the relation between the effects of growth substance and the development of crown gall. The work here reported falls under four headings: (1) A survey of responses to inoculation indicating the presence of growth substance in abnormal amount, (2) estimation of amount of growth substance in inoculated and uninoculated tissues, (3) production of growth substance by bacteria in culture, and (4) determination of the effect of an external source of growth substance upon the proliferation of tissues inoculated with an attenuated strain of the crown gall organism. The activities of attenuated and virulent strains have been compared when feasible. A preliminary report (*23*) has already appeared.

MATERIALS AND METHODS

Virulent (A6) and attenuated (A6–6), single-cell sister crown gall cultures described by Hendrickson, Baldwin, and Riker (*9*) were em-

ployed. Numerous inoculations have been made into tomato, *Sedum*, *Bryophyllum*, and *Kalanchoe*. The attenuated culture has consistently induced some proliferation, although it is very slight on tomato and is always less than that induced by the virulent strain on the other plants.

Lycopersicum esculentum Mill. was employed because of ease of propagation and the short time required for development of galls following inoculation. *Bryophyllum pinnatum* (L.) Kurtz and *Kalanchoe diagremontiana* Hamet et Perrier were used because they produce relatively large galls with the attenuated strain and have more completely dormant axillary buds than the tomato.

Avena coleoptile tests were made in a dark room at 23° to 25° C., with over 90 percent relative humidity. The oats, supplied by Dr. H. L. Shands, were of a genetically stable variety designated "State's Pride, Pedigree 7–7." [4] When the coleoptile reached a length of 2.5 to 3.0 cm they were ready for use. The first leaf was pulled almost out and cut off a few millimeters above the coleoptile tip. The seedlings were placed in individual holders with their roots in water. The test itself was carried out in two ways.

For detecting growth substance in inoculated tissues, Went's method *(43a)* was used. Tomato seedlings (about 10 cm tall) were inoculated at three points on the stem by means of needle punctures. Control plants received sterile needle punctures. After the desired interval of time 1-cm sections, each including an inoculation or control puncture, were cut from the stems and placed upright on blocks of 3-percent agar having the dimensions 1.5 by 4 by 4 mm. After 2.5 hours the sections were removed and the agar blocks cut into four equal parts. These were placed unilaterally on decapitated oat coleoptiles. The curvatures produced in 2.5 hours were recorded and translated into "plant units" (the amount of growth substance applied in one agar block to give an angle of 1°, cf. *(3)*). Based upon the size of blocks employed in these experiments, one plant unit is equal to 0.3 *Avena* unit.

A second method, patterned after that of Brecht *(4)*, was used for the detection of growth substance in culture fluids. The acidified culture was extracted several times with chloroform, the combined extracts equaling twice the volume of the culture. The chloroform was then evaporated off and the residue taken up in a small amount of water. An equal weight of lanolin was then mixed with the water to form a stiff paste. This was applied along one side of decapitated coleoptiles for their full length. Readings on the curvatures produced were made over a 24-hour period.

In some of the experiments beta-indole-acetic acid and beta-indole-propionic acid in the form of lanolin-water emulsions were used. Low concentrations of chemicals were mixed with the water before making the emulsion. High concentrations were first mixed with the lanolin.

RESPONSES OF INOCULATED PLANTS SUGGESTING THE PRESENCE OF GROWTH SUBSTANCE

EPINASTY OF LEAF PETIOLES

Epinasty in the lower leaves of young tomato plants bearing several well-developed galls was studied in a preliminary experiment. Two tomato plants about 15 cm tall were inoculated at five points along

[4] This variety gave approximately the same sensitivity as the Victory oats generally employed in plant hormone work.

the stem by means of needle punctures, one with the virulent strain, and one with the attenuated strain. A third received sterile punctures. Nineteen days later galls were evident on the plant inoculated

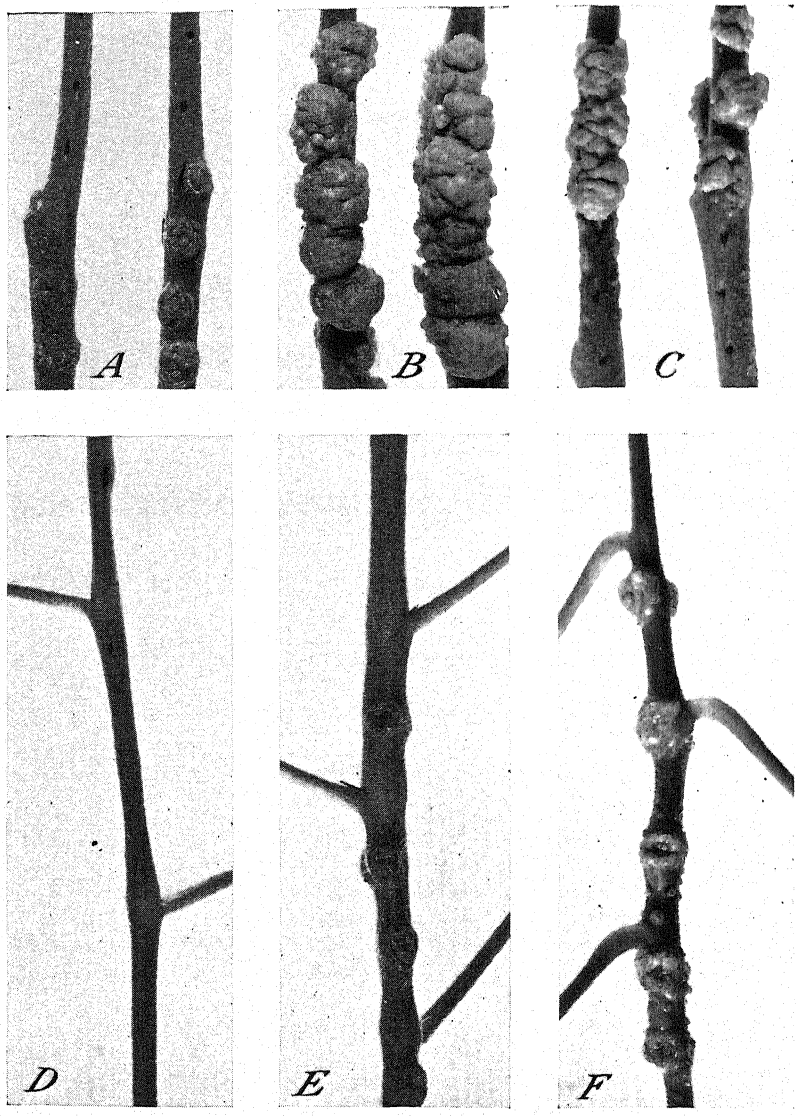


FIGURE 1.—Stimulation of tissues inoculated with the attenuated crown gall bacteria, and production of epinasty following inoculation with the virulent strain. Decapitated tomato plants six weeks after inoculation: *A*, Three control punctures above and three attenuated-strain inoculations below; *B*, three virulent-strain inoculations above and three attenuated-strain inoculations below; *C*, three virulent-strain inoculations above and three control punctures below. Intact tomato plants 19 days after inoculation at five points along the stem: *D*, Plant with control punctures; *E*, inoculated with the attentuated strain; *F*, inoculated with the virulent strain.

with the virulent strain, and the petioles originating near the galls were bent sharply downward (fig. 1, *F*). The plant inoculated with the attenuated strain (fig. 1, *E*) bore small swellings at the points of

inoculation and the petioles originating near them maintained approximately the same angle as those of the control plant (fig. 1, *D*).

Similar results have been observed in a considerable variety of experiments. The results from two representative trials are given in table 1. Here it appears that, while the virulent strain increased epinasty greatly in 14 days, the attenuated strain had no apparent influence upon it.

TABLE 1.—*Epinasty of tomato petioles following crown gall inoculations* [1]

Inoculant	Extent of epinasty after number of days indicated					
	Trial No. 1			Trial No. 2		
	0 days	7 days	14 days	0 days	7 days	14 days
	Degrees	*Degrees*	*Degrees*	*Degrees*	*Degrees*	*Degrees*
Uninoculated	57	66	76	59	69	75
Attenuated strain	56	65	74	62	67	65
Virulent strain	63	79	117	64	81	107

[1] Plants 6 inches high were inoculated 4 or 5 times, once between each node. Controls received sterile punctures. The degrees given are the averages of 30 axillary angles, 5 on each of 6 plants.

It was noted on numerous occasions that tomato plants bearing a single inoculation with the virulent strain exhibited very little epinasty. This suggested a relation between the number of inoculations and the degree of epinasty produced, which was tested on four lots of five plants each, bearing different numbers of inoculations. The results in table 2 show that, with an increase in the number of inoculations, there was in general an increase in the amount of epinasty induced. This is partly because more petioles were near galls on plants inoculated in a greater number of internodes, but not entirely, since the petioles immediately below the uppermost inoculation showed greater epinasty when additional inoculations were made in the internodes farther down the stem.

TABLE 2.—*Increase in epinasty of tomato petioles following increase in number of inoculations with the virulent strain* [1]

Inoculations (number)	Extent of epinasty shown by petioles in position indicated			Average axillary angle
	First	Second	Third	
	Degrees	*Degrees*	*Degrees*	*Degrees*
1	90	84	90	88
2	90	105	103	99
4	106	104	101	103
8	110	113	125	116

[1] The plants were inoculated once in each of 1, 2, 4, and 8 internodes respectively, beginning at the top (first) and proceeding downward. The degrees given are averages of corresponding axillary angles of 5 plants 3 weeks after inoculation.

INITIATION OF ADVENTITIOUS ROOTS

The initiation of roots in conjunction with crown gall development was observed on several species. Figure 2 shows *Kalanchoe* plants 1 month after treatment. No roots appear on the control plant

(*D*) or on the plant inoculated with the attenuated strain (*E*). On the plant inoculated with the virulent strain (*F*), numerous adventi-

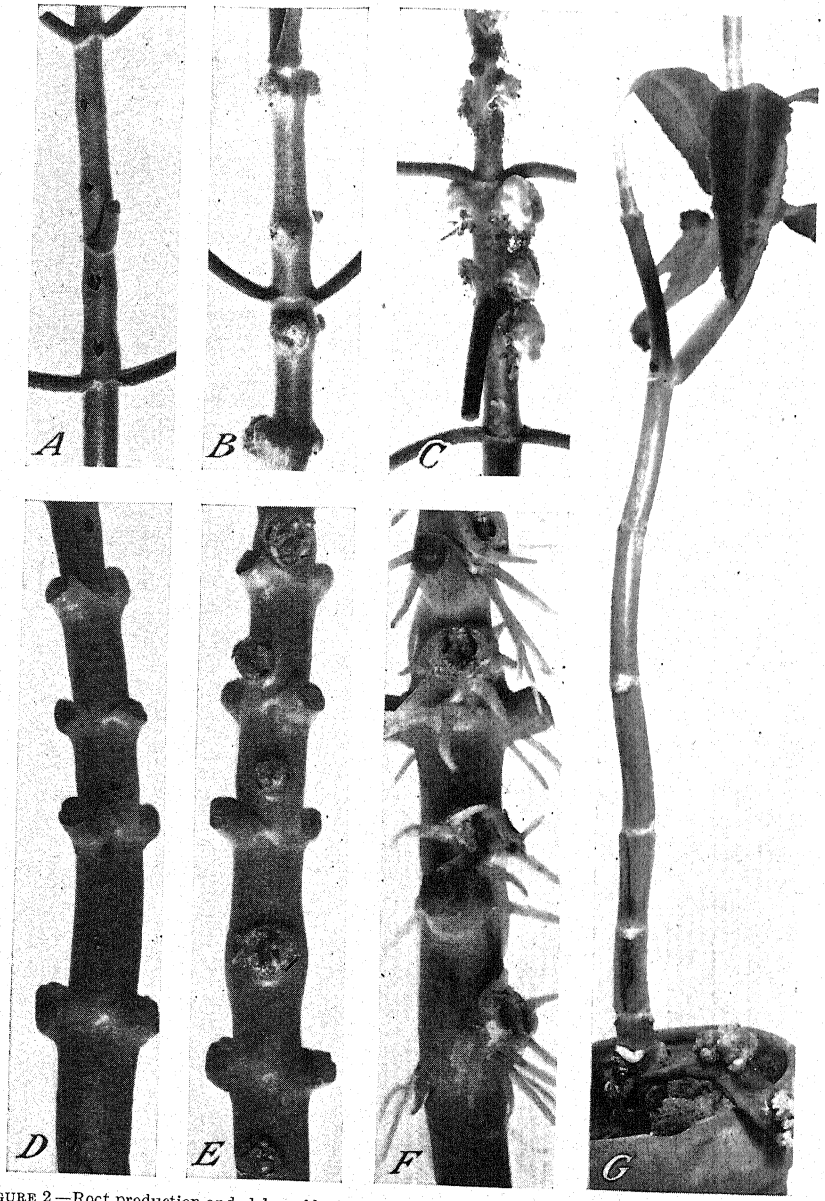


FIGURE 2.—Root production and delay of leaf abscission resulting from inoculations with crown gall bacteria. *Bryophyllum pinnatum* plants: *A*, Uninoculated; *B*, inoculated with the attenuated strain; *C*, inoculated with the virulent strain. *Kalanchoe diagremontiana* plants: *D*, Uninoculated; *E*, inoculated with the attenuated strain; *F*, inoculated with the virulent strain. *Kalanchoe* leaf (*G*) several months after inoculation with the virulent strain; leaves of eight younger nodes have been shed while inoculated leaf at the base remains firmly attached.

tious roots developed about the inoculations, sometimes at a distance from the inoculated tissue. Likewise in figure 3, *A*, where a decapi-

tated plant was inoculated on the cut surface, the roots appeared on the internode several centimeters below the gall. Such results have been obtained with this host repeatedly. These roots are not to be confused with those arising from the hairy root organism, *Phytomonas rhizogenes*.

Root initiation was obtained when *Bryophyllum* was inoculated with the virulent strain, but there was little subsequent development. Figure 2 shows three plants approximately 1 month after treatment. Roots appeared abundantly from the lower margins of the galls induced by the virulent strain (*C*), while none appeared about the swellings induced by the attenuated strain (*B*) or on the control plants (*A*).

Root initiation on inoculated tomato plants was quite abundant. In one experiment plants about 12 cm tall were inoculated in the first five internodes by means of a needle puncture, six with the virulent strain and six with the attenuated strain. Six more received sterile punctures. The numbers of root primordia appearing on the stems were recorded at the time of inoculation and after 1 and 2 weeks had passed. After 2 weeks normal root initiation on the stems tends to equalize differences between inoculated and uninoculated plants. The results of two representative trials appear in table 3. Plants inoculated with the virulent strain produced many more root primordia than either the controls or those inoculated with the attenuated strain.

TABLE 3.—*Initiation of root primordia on stems of tomato plants inoculated with crown gall bacteria* [1]

Inoculant	Root primordia on stems after number of days indicated					
	Trial No. 1			Trial No. 2		
	0 days	7 days	14 days	0 days	7 days	14 days
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
Uninoculated	0	2	6	0	0	6
Attenuated strain	0	2	5	0	1	21
Virulent strain	1	13	44	0	14	61

[1] Plants were inoculated 4 or 5 times, once for each internode. The figures given are the average number of root primordia appearing on 6 plants.

STIMULATION OF THE CAMBIUM

Cambial stimulation was studied in tomato plants which were decapitated when they were about 20 cm tall at a point where the stem was about 3 mm in diameter. Lots of four plants each were thus prepared. To the cut stem tip of one lot the virulent strain was applied, to that of another the attenuated strain was applied, and that of a third was left untreated. Two weeks later gall tissue was visible on the plants inoculated with the virulent strain while none was apparent on the others. The internode below the gall had increased in thickness at about the same rate as the corresponding internodes of whole plants. However, on the other plants the internode below the point of decapitation remained about the same in diameter as when the cut was made. One month after inoculation the stem thickness

was measured, two readings being taken at right angles to each other. The average of four pairs of readings was 3.3 mm for the uninoculated plants, 4.5 mm for those inoculated with the attenuated strain, and

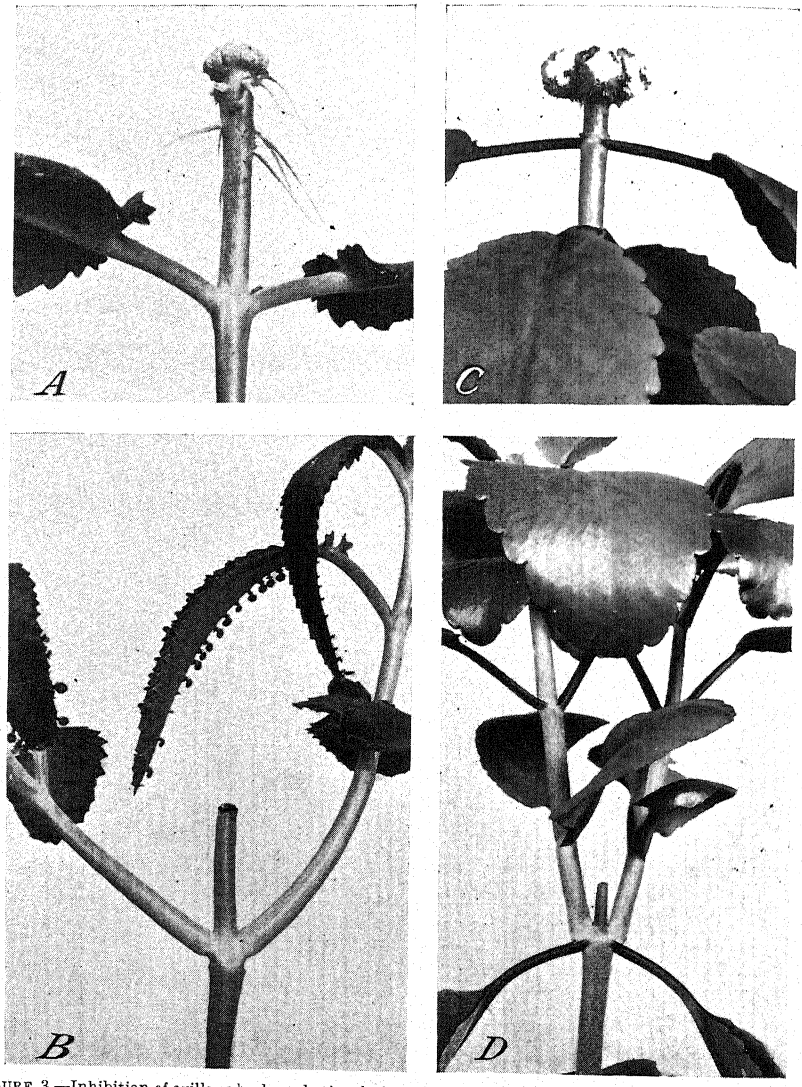


FIGURE 3.—Inhibition of axillary buds and stimulation of cambial activity resulting from inoculation wit crown gall bacteria. *A*. Decapitated *Kalanchoe* plant inoculated on the cut surface with the virulent strain. Axillary buds have failed to develop and the decapitated internode has increased in thickness. *B*. Decapitated *Kalanchoe* plant, uninoculated, showing shoots which have developed from axillary buds of the uppermost node. (The leaves have been shed from this node.) *C* and *D*. Decapitated *Bryophyllum* plants treated like the plants in *A* and *B*, respectively, and showing similar results. Photographed 7 weeks after treatment.

6.0 mm for those inoculated with the virulent strain. Similar results were obtained in a second trial. Stimulation of cambium in decapitated internodes of *Kalanchoe* and *Bryophyllum* also was obtained following inoculation with the virulent strain of crown gall bacteria

(fig. 3). Sliding-microtome sections of the *Kalanchoe* plants showed a solid cylinder of secondary xylem in the inoculated plant but apparently no secondary xylem in the uninoculated plant (fig. 4). Freehand sections of both sets of material revealed that, although some

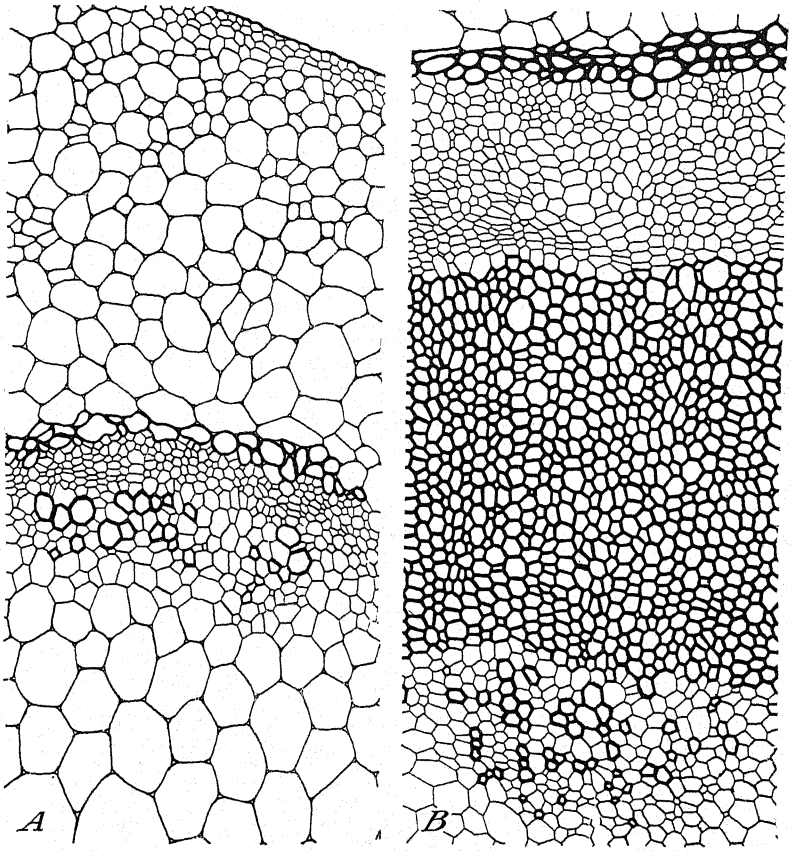


FIGURE 4.—Camera-lucida drawings of portions of vascular cylinders of decapitated *Kalanchoe* plants 7 weeks after treatment: *A*, an uninoculated plant showing no secondary xylem; *B*, a plant inoculated on the cut surface with the virulent strain of crown gall bacteria, showing a broad band of heavy-walled secondary xylem. These sections were made a few millimeters below the tips of plants *A* and *B* of figure 3.

increase in size of the pith cells had occurred, the greater part of the increase in thickness resulted from activity of the cambium.

INHIBITION OF BUD DEVELOPMENT

With *Kalanchoe* and *Bryophyllum* the inhibition of development of the axillary buds was clear-cut in repeated trials with plants decapitated and inoculated with the virulent strain on the cut surface (fig. 3, *A*, *C*). In plants decapitated but not inoculated (fig. 3, *B*, *D*) the axillary buds of the uppermost remaining node developed into shoots.

Inhibition of axillary buds following decapitation was similarly obtained in the vicinity of inoculations in repeated trials with tomato, but only when removal of the part of the plant above the inoculations was delayed until galls were well developed.

Evidence of inhibition of development of adventitious buds was obtained with decapitated tomato plants. In the first trial three lots of eight plants each were employed. Plants inoculated on the cut surface with the virulent strain developed abundant gall tissue

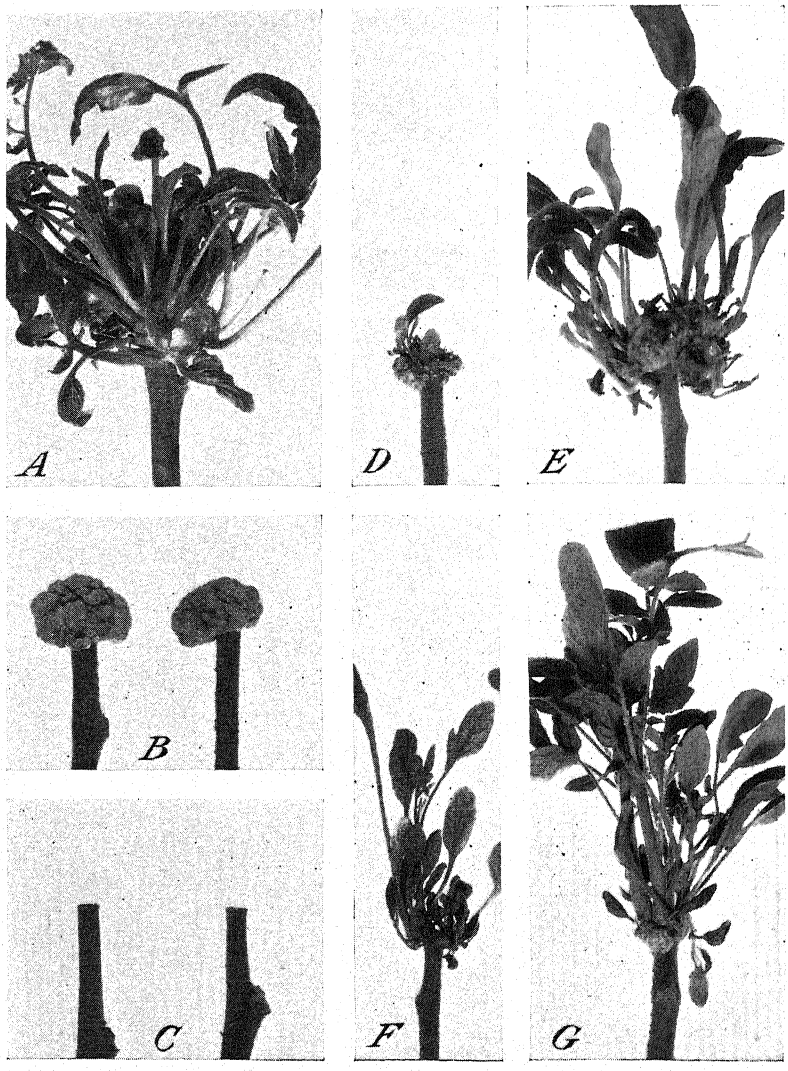


FIGURE 5.—Inhibition of development of adventitious shoots on decapitated tomato plants inoculated with virulent crown gall bacteria. Axillary buds not removed: *A*, Inoculated with the attenuated strain; *B*, inoculated with the virulent strain; *C*, uninoculated. Axillary buds removed: *D*, Inoculated with the virulent strain; *E*, inoculated with the attenuated strain; *F*, uninoculated; *G*, inoculated with a mixture of the virulent and attenuated strains.

but no adventitious buds (fig. 5, *B*). Plants inoculated on the cut surface with the attenuated strain developed numerous adventitious buds and shoots and, later, large masses of green gall tissue at the cut surface (fig. 5, *A*). Uninoculated plants developed no callus or adventitious buds at the cut surface (fig. 5, *C*.) A second trial was

made on decapitated plants from which, in addition, all axillary buds had been removed. This treatment resulted in a greater tendency to produce adventitious buds, which appeared on all of the plants. However, on plants inoculated with the virulent strain these remained very small (fig. 5, *D*). On plants inoculated with the attenuated strain large shoots and later large masses of green gall tissue developed (fig. 5, *E*). Uninoculated plants developed shoots but no gall tissue (fig. 5, *F*). Plants inoculated with a mixture of virulent and attenuated strains produced two kinds of gall tissue, one pale and free of buds and the other green and bearing numerous adventitious shoots (fig. 5, *G*).

The effect of the attenuated strain on development of axillary buds was not determined on *Kalanchoe* and *Bryophyllum* by the above method because inoculations made on the cut surfaces of decapitated plants consistently failed to develop galls. Stem-puncture inoculations, however, produce good-sized galls on these plants, and it has been observed that axillary buds in the vicinity of the galls frequently develop into short shoots even when the top of the plant is not removed. This has never been observed in the dozens of plants inoculated with the virulent strain during the course of this study. These results indicate that the attenuated strain not only fails to inhibit bud development but even stimulates it.

The mechanism of this stimulation is not known. Among the possibilities are (1) a specific shoot-stimulating substance, (2) isolation of the axillary buds from influence of the apical bud by distortion of conducting tissues, and (3) removal of growth substance or other growth inhibitors by the inoculated tissues.

DELAY OF PETIOLE ABSCISSION

Delay of abscission of leaves inoculated with crown gall was conspicuous in *Kalanchoe*. In one case a lower leaf bearing two galls about 1.5 cm in diameter persisted while the plant had increased in height and had shed the leaves of eight higher nodes (fig. 2, *G*).

Similar effects were observed on tomato. For example, six plants each were inoculated with the virulent and attenuated strains by means of shallow needle punctures on the upper surface of several petioles just below the first leaflet. Control plants were treated similarly, using a sterile needle. Two weeks after inoculation swellings were visible at the inoculated points. At this time the parts distal to the swellings were cut off. Virulent and attenuated strains of the crown gall bacteria were in this case about equally effective in inhibiting abscission (table 4).

TABLE 4.—*The inhibiting effect of crown gall on abscission of leaf petioles in tomato* [1]

Inoculant	Treated petioles	Treated petioles remaining attached after—			
		14 days	19 days	33 days	43 days
	Number	*Percent*	*Percent*	*Percent*	*Percent*
Uninoculated	28	64	25	0	0
Attenuated strain	64	100	100	67	39
Virulent strain	31	100	93	58	35

[1] Petioles were inoculated just below the first leaflet 2 weeks previous to removing the distal portion of the leaf.

The effect of inoculation with crown gall bacteria on petiole abscission in *Bryophyllum* was tested on lots of seven plants each. One petiole of five or six pairs on each plant was inoculated at the base of the leaf blade. The other member of each pair received a control puncture. Six weeks after inoculation the leaf blades were removed by severing just above the point of inoculation. At this time small galls (2 to 5 mm) had developed from the inoculations made with the virulent strain. Inoculations with the attenuated strain had produced swellings only slightly larger than those on the 61 control petioles. Three days after the removal of the leaf blades all of the control petioles had been shed. Of the 32 petioles inoculated with the attenuated strain 8 remained attached. All 29 of the petioles inoculated with the virulent strain remained attached and were still attached after 2 weeks. At this time two additional petioles inoculated with the attenuated strain had been shed. In this case the virulent strain was more effective than the attenuated strain in preventing abscission. Other trials on *Bryophyllum* gave similar results.

DETERMINATION OF GROWTH SUBSTANCE IN INOCULATED TISSUE

Although the foregoing experiments furnish strong evidence for the production, either by the bacteria or by the host cells, of growth substance in inoculated tissues, more direct evidence was sought. Went's *Avena* test was employed as described in the section on materials and methods.

In one trial determinations were made on one inoculated and one uninoculated tomato seedling 3 days after inoculation, and on similar plants, 6 and 9 days after inoculation. The amount of growth substance detected, expressed in plant units, appears in table 5. Another trial was made in which three seedlings were used for each determination after intervals of 3, 6, 8, and 10 days. The results were similar to those of the first trial. More growth substance was obtained from inoculated than from the uninoculated tissue.

Unsuccessful trials were made by means of Went's *Avena* test with tissue from well-developed galls. No significant amount of growth substance was detected. Likewise, attempts to extract large crown galls with chloroform and alcohol to secure a growth substance have failed. The details are omitted because of the negative results.

TABLE 5.—*Production of growth substance in tomato seedlings inoculated with crown gall bacteria* [1]

Days after inoculation	Production of growth substance per seedling of—	
	Uninoculated plants	Inoculated plants
	Plant units [2]	*Plant units* [2]
3	53	86
6	16	143
9	17	276

[1] Determinations were made by means of Went's *Avena* test on 3 1-cm stem sections from a single seedling, each bearing an inoculation with the virulent strain or a control puncture.

[2] The amount of growth substance applied in 1 agar block to give an angle of 1° (equal to 0.3 *Avena* unit in this case; thus 276 plant units equal 83 *Avena* units).

PRODUCTION OF GROWTH SUBSTANCE IN CULTURE

Growth-substance production by crown gall bacteria in media was studied in a preliminary way. Such substances were found in cultures on peptone medium containing tryptophane by Brown and Gardner (*6*) and by Link, Wilcox, and Link (*22*). In view of reports of similar action by various other micro-organisms (*3*) it seemed desirable to make a comparison between pathogenic and nonpathogenic organisms with respect to this character.

Several preliminary experiments have been completed with the virulent and attenuated strains of crown gall bacteria and with a single-cell culture of *Bacillus radiobacter* Beij. and Van Deld., in beef broth containing peptone and tryptophane. Growth substance was extracted from the cultures with chloroform, recovered by evaporation, and incorporated into a lanolin-water paste. The activities of the various preparations were tested on oat coleoptiles after Brecht's method (14). Some growth substance was obtained from cultures of all three organisms and also from the unseeded medium. Only about four times as much was obtained from the virulent crown gall cultures as from the nonpathogenic *B. radiobacter* culture. No consistent difference in the production of growth substance has thus far been established between the virulent and attenuated cultures of crown gall bacterial.

EFFECT OF GROWTH SUBSTANCE ON TISSUE INOCULATED WITH THE ATTENUATED STRAIN

The relation of growth substance to the development of tissue about inoculations with the attenuated culture was studied. It appeared that, if the failure of the attenuated strain of crown gall bacteria to induce galls on tomato were associated with the absence of growth substance the application of growth substance should result in increased proliferation. Tests were made with growth substance from several sources.

GROWTH SUBSTANCE FROM THE EXPANDING FOLIAGE OF THE HOST

When inoculated into tomato plants the attenuated strain may have some effect, as already seen in figure 1, *E*. Swellings were induced that were distinguishable from those resulting from control punctures, possibly owing to the growth substance which normally originates in the expanding foliage of the plant, which passes downward into the inoculated tissues, and there increases proliferation. To test this suggestion, three inoculations were made with the attenuated strain in each of four decapitated and four whole tomato plants. Control plants received sterile punctures. Ten days later inoculations on decapitated plants were indistinguishable from control punctures while those on whole plants showed swellings distinctly greater than those resulting from control punctures. Similar results were obtained in a second trial on tomato employing lots of eight plants each. In this trial slight swellings appeared after 6 weeks on some of the decapitated plants, but the largest of these (fig. 1, *A*) were smaller than the smallest of those on the whole plants. In some cases the inoculations with the attenuated culture on decapitated plants was little, if any, larger than the control puncture.

The effect of expanding foliage on inoculations with the attenuated strain was also tested on *Bryophyllum*. Five plants ranging from 12 to 20 cm in height were inoculated with this strain five times in the upper part of the stem. Five more were inoculated in the same way but after they had been decapitated and defoliated in the region inoculated. Galls developed from all of the inoculations in the whole plants, while in the decapitated plants gall development took place in less than half of the cases and was slower. The stimulating effect of the foliage was quite evident.

GROWTH SUBSTANCE FROM GALLS INDUCED BY THE VIRULENT STRAIN

Experiments were made to determine the effect of growth substance from active galls upon tissues inoculated with the attenuated strain. Three lots each containing six tomato plants about 25 cm high were decapitated and the leaves and axillary buds of the two nodes next below were removed, leaving a bare stub approximately 10 cm in length. Six puncture treatments were made approximately 1 cm apart in the denuded stubs as follows: In the first lot, three control punctures at the top and three inoculations with the attenuated strain below; in the second lot, three inoculations with the virulent strain at the top and three with the attenuated strain below; and in the third lot, three inoculations with the virulent strain at the top and three control punctures below. Six weeks after inoculation, galls developing from the inoculations with the virulent strain had attained diameters ranging from 1 to 2 cm. No swellings developed at the control punctures made below the virulent strain inoculations. From 18 inoculations made with the attenuated strain below control punctures there developed only 4 very small proliferations (1 to 5 mm in diameter), while from an equal number of inoculations made below inoculations with the virulent strain there arose 4 large (10 to 15 mm in diameter) and 5 small (5 mm in diameter) galls.

Similar results were obtained in two other trials involving lots of four and eight plants per treatment, respectively. Results are illustrated in figure 1. In both of these trials the inoculations with the attenuated culture were made 3 days before those with the pathogenic culture in order to reduce the possibility of contamination. These experiments indicate that, when some substance from the tissue inoculated with the virulent strain diffuses down the stem, inoculations with the attenuated strain are stimulated until they equal in size those of the virulent strain. Here, as in other inoculations on tomato with these two cultures, the galls from virulent cultures were without chlorophyll while those from attenuated cultures were green.

BETA-INDOLE-ACETIC ACID

The effect of beta-indole-acetic acid on tissues inoculated with the attenuated strain was tested. Decapitated tomato stems were inoculated at three points near the top with the attenuated strain and the growth substance was applied in lanolin-water paste to the tip of the stub and renewed at weekly intervals during the experiment. Three trials made with lanolin-water paste containing 0.05 mg of beta-indole-acetic acid per gram gave only negative results. The fourth and fifth trials were made with a high concentration of beta-

indole-acetic acid (30 mg per gram of paste). In the fourth trial the axillary buds were removed from the plants. Four weeks after inoculation the stems of plants treated with lanolin-water paste containing beta-indole-acetic acid had grown until their diameters were twice those of the other plants. The results were confused by the appearance of adventitious shoots at the inoculations on the control plants and subsequently of smooth, dark-green galls, possibly conditioned by growth substance originating in the adventitious shoots. Nevertheless, there was apparently a slight stimulation in plants treated with the acid over untreated plants.

A fifth trial with lots of 10 tomato plants each was carried out in the same way except that the axillary buds were not removed and adventitious shoots did not appear. After 10 weeks the galls were removed and weighed. For plants treated with lanolin-water paste containing beta-indole-acetic acid the average weight of gall tissue per plant was 1.04 g. For control plants treated with lanolin-water paste containing no growth substance it was 0.70 g.

These results indicate that, under the conditions of these experiments, beta-indole-acetic acid in high concentration is only slightly effective in stimulating proliferation of tissues inoculated with the attenuated strain, being less effective in this respect than substances diffusing from active galls and from the host plant foliage.

DISCUSSION

It appears from the evidence presented that growth substance is associated with crown gall tissue. Also, it is apparently a limiting factor in the development of galls on tomato by the attenuated strain used.

Several recent writers (*6*, *12*, *22*) have dealt with the parallel between the response of beans to beta-indole-acetic acid and crown gall on various plants. These workers have been very guarded about conclusions. Link, Wilcox, and Link (*22*) report that galls are the result of a causal complex, of which beta-indole-acetic acid possibly is one constituent. The identity of this substance was based on a color reaction with a crude extract and is open to question.

Various nonpathogenic as well as pathogenic organisms produce beta-indole-acetic acid on suitable peptone medium. It has been considered (*22*) that gall production may be initially conditioned by "factors determining infection" rather than by specificity of the chemical mechanism involved. In this connection it is interesting that the attenuated crown gall culture multiplies within tomato stem tissues (*25*) for the common incubation period at approximately the same rate as the virulent cultures. Thus during the incubation period the plant has apparently no bacteriostatic effect on the causal organisms.

In the production of proliferation on beans, beta-indole-acetic acid has been commonly employed at a concentration of 30 mg per gram of lanolin (*6*, *12*, *22*). One-tenth this concentration gave little if any response. As employed by the present writers one application of this paste amounted to one twenty-fifth of a gram and therefore contained 30,000,000 *Avena* units (*3*). During this investigation the greatest amount of growth substance obtained by the *Avena* coleoptile technique from three crown gall inoculations was 83 *Avena* units (table 5).

Yet substances diffusing from three such inoculations produced greater stimulation of tissues inoculated with the attenuated strain than the high concentration of beta-indole-acetic acid employed.

Responses of plants like bean, sunflower, *Coleus*, and *Impatiens* to beta-indole-acetic acid do not parallel their responses to crown gall bacteria (*28*).

Another item for consideration is the number of bacteria involved. In order to obtain enough growth substance to produce significant proliferation with present technique a relatively large quantity of culture must be extracted—a quantity containing a great many times the number of bacteria present in a comparable crown gall.

In view of the evidence now available it seems better to reserve judgment about beta-indole-acetic acid playing a major role in the development of crown gall.

As was pointed out by Leonian (*3, review*) galls may be the result of excessive production of growth substance by the host tissues under the influence of the bacteria rather than the result of growth substance furnished by the pathogen to the host plant. This view finds support in the fact that substances diffusing from the host plant foliage and from active galls are alike in that they stimulate proliferation of tissues inoculated with the attenuated strain much more than do applications of beta-indole-acetic acid. Where they have been identified, growth substances obtained from higher plants have been of the auxin-a or auxin-b type, while those obtained from cultures of bacteria have been of the heteroauxin type, (*3, 38*). It therefore seems probable that the substance coming from the active gall is, like that from the host plant foliage, of the auxin-a or auxin-b type.

SUMMARY

Plants inoculated with a virulent strain of *Phytomonas tumefaciens* exhibit, in addition to gall development, responses that suggest an increase in amount of growth substances present. These responses include (1) increased epinasty of leaf petioles, (2) increased initiation of adventitious roots, (3) stimulated cambial activity, (4) inhibited development of certain buds, and (5) delayed abscission of senescent leaves. An attenuated sister strain was less effective in inducing most of these effects as well as in bringing about gall development.

Adventitious shoots were stimulated on decapitated tomato plants by inoculating the wound with the attenuated culture.

Greater amounts of growth substance were detected by the Went's *Avena* test in inoculated than in comparable uninoculated tissues of tomato seedlings. The amounts detected were equivalent to only a tiny fraction of that commonly used in the beta-indole-acetic acid treatment for production of proliferations on bean.

Preliminary experiments failed to establish a relation between ability of virulent and attenuated cultures to produce growth substance in culture and ability to induce galls in plants.

Growth substance from expanding foliage provided some stimulation to tissue inoculated with the attenuated culture. Such substance from gall tissue induced by a virulent strain stimulated strongly the tissue inoculated by the attenuated strain. Beta-indole-acetic acid was only slightly effective in this respect.

The probable origin and chemical nature of the growth substance or substances involved are discussed. It is concluded that the chief growth substance thus far detected in crown gall is more probably of the auxin-a or auxin-b type than of the heteroauxin type, and that it is more likely a product of the host cells under the influence of the bacteria than a direct bacterial metabolic product.

LITERATURE CITED

(1) AVERY, G. S., JR., BURKHOLDER, P. R., and CREIGHTON, H. B.
1937. PRODUCTION AND DISTRIBUTION OF GROWTH HORMONE IN SHOOTS OF AESCULUS AND MALUS, AND ITS PROBABLE ROLE IN STIMULATING CAMBIAL ACTIVITY. Amer. Jour. Bot. 24: 51–58, illus.

(2) BAUGUESS, L. C.
1935. PLANT RESPONSES TO SOME INDOLE DERIVATIVES. (Abstract) Amer. Jour. Bot. 22: 910–911.

(3) BOYSEN-JENSEN, P.
1936. GROWTH HORMONES IN PLANTS. Authorized English transl. . . . Transl. and rev. by G. S. Avery, Jr., and P. R. Burkholder, H. B. Creighton, and B. A. Scheer. 268 pp., illus. New York and London. [Reviewed by L. H. Leonian in Phytopathology 27: 117–118. 1937.]

(4) BRECHT, F.
1936. DER EINFLUSS VON WUCHSTOFF- UND SÄUREPASTEN AUF DAS WACHSTUM VON AVENA UND HELIANTHUS KEIMLINGEN UND SEINE ABHÄNGIGKEIT VOM SAUERSTOFFGEHALT DER LUFT. Jahrb. Wiss. Bot. 82: [581]–612, illus.

(5) BROWN, N. A.
1929. THE TENDENCY OF THE CROWN-GALL ORGANISM TO PRODUCE ROOTS IN CONJUNCTION WITH TUMORS. Jour. Agr. Research 39: 747–766, illus.

(6) ——— and GARDNER, F. E.
1936. GALLS PRODUCED BY PLANT HORMONES, INCLUDING A HORMONE EXTRACTED FROM BACTERIUM TUMEFACIENS. Phytopathology 26: 708–713, illus.

(7) CROCKER, W.
1929. A DELICATE METHOD OF DETECTING ILLUMINATING GAS IN A GREENHOUSE. Florists' Exch. 70: 15, 54, illus. Also in Boyce Thompson Inst. Prof. Paper 1: 81–85.

(8) ——— ZIMMERMAN, P. W., and HITCHCOCK, A. E.
1932. ETHYLENE-INDUCED EPINASTY OF LEAVES AND THE RELATION OF GRAVITY TO IT. Contrib. Boyce Thompson Inst. Plant Research 4: 177–218, illus.

(9) HENDRICKSON, A. A., BALDWIN, I. L., and RIKER, A. J.
1934. STUDIES ON CERTAIN PHYSIOLOGICAL CHARACTERS OF PHYTOMONAS TUMEFACIENS, PHYTOMONAS RHIZOGENES, AND BACILLUS RADIOBACTER. II. Jour. Bact. 28: 597–618, illus.

(10) HITCHCOCK, A. E.
1935. INDOLE-3-N-PROPIONIC ACID AS A GROWTH HORMONE AND THE QUANTITATIVE MEASUREMENT OF PLANT RESPONSE. Contrib. Boyce Thompson Inst. Plant Research 7: 87–95, illus.

(11) ———
1935. TOBACCO AS A TEST PLANT FOR COMPARING THE EFFECTIVENESS OF PREPARATIONS CONTAINING GROWTH SUBSTANCES. Contrib. Boyce Thompson Inst. Plant Research 7: 349–364, illus.

(12) KRAUS, E. J., BROWN, N. A., and HAMNER, K. C.
1936. HISTOLOGICAL REACTIONS OF BEAN PLANTS TO INDOLEACETIC ACID. Bot. Gaz. 98: 370–420, illus.

(13) LAIBACH, F.
1933. WUCHSSTOFFVERSUCHE MIT LEBENDEN ORCHIDEENPOLLINIEN. Ber. Deut. Bot. Gesell. 51: 336–340, illus.

(14) ———
1935. ÜBER DIE AUSLÖSUNG VON KALLUS- UND WURZELBILDUNG DURCH BETA-INDOLYLESSIGSÄURE. Ber. Deut. Bot. Gesell. 53: 359–364, illus.

(15) LAIBACH, F. and FISCHNICH, O.
1935. ÜBER EINE TESTMETHODE ZUR PRÜFUNG DER KALLUSBILDENEN WIRKUNG VON WUCHSSTOFFPASTEN. Ber. Deut. Bot. Gesell. 53: 469–477, illus.
(16) ——— MAI, G., and MÜLLER, A.
1934. ÜBER EIN ZELLTEILUNGSHORMON. Naturwissenschaften 22: 288.
(17) LARUE, C. D.
1935. THE RÔLE OF AUXIN IN THE DEVELOPMENT OF INTUMESCENCES ON POPLAR LEAVES; IN THE PRODUCTION OF CELL OUTGROWTHS IN THE TUNNELS OF LEAF-MINERS; AND IN THE LEAF-FALL IN COLEUS. (Abstract) Amer. Jour. Bot. 22: 908.
(18) LEVINE, M.
1919. STUDIES ON PLANT CANCERS—I. THE MECHANISM OF THE FORMATION OF THE LEAFY CROWN GALL. Bull. Torrey Bot. Club 46: 447–452, illus.
(19) ———
1923. STUDIES ON PLANT CANCERS V. LEAFY CROWN GALLS ON TOBACCO PLANTS RESULTING FROM BACTERIUM TUMEFACIENS INOCULATIONS. Phytopathology 13: [107]–116, illus.
(20) ———
1924. CROWN GALL ON BRYOPHYLLUM CALYCINUM. Bull. Torrey Bot. Club. 51: 449–456, illus.
(21) ——— and CHARGAFF, E.
1937. THE RESPONSE OF PLANTS TO CHEMICAL FRACTIONS OF BACTERIUM TUMEFACIENS. Amer. Jour. Bot. 24: 461–472, illus.
(22) LINK, G. K. K., WILCOX, H. W., and LINK, A. D.
1937. RESPONSES OF BEAN AND TOMATO TO PHYTOMONAS TUMEFACIENS, P. TUMEFACIENS EXTRACTS, β-INDOLEACETIC ACID, AND WOUNDING. Bot. Gaz. 98: 861–867, illus.
(23) LOCKE, S. B, RIKER, A. J., and DUGGAR, B. M.
1937. A GROWTH HORMONE IN THE DEVELOPMENT OF CROWN GALL. (Abstract) Phytopathology 27: 134.
(24) LYNEIS, M. M., RIKER, A. J., and LOCKE, S. B.
1936. COMPARATIVE PHYSIOLOGY OF PATHOGENIC AND NON-PATHOGENIC CROWN-GALL BACTERIA. (Abstract) Phytopathology 26:100.
(25) NĚMEC, B.
1930. BAKTERIELLE WUCHSSTOFFE. Ber. Deut. Bot. Gesell. 48: 72–74.
(26) RIKER, A. J.
1923. SOME MORPHOLOGICAL RESPONSES OF THE HOST TISSUE TO THE CROWNGALL ORGANISM. Jour. Agr. Research 26: 425–435, illus.
(27) ——— and BERGE, T. O.
1935. ATYPICAL AND PATHOLOGICAL MULTIPLICATION OF CELLS APPROACHED THROUGH STUDIES ON CROWN GALL. Amer. Jour. Cancer 25: 310–357, illus.
(28) ——— and NAGY, R.
1938. CELL STIMULATION BY CHEMICALS IN RELATION TO CROWN GALL. (Abstract) Phytopathology 28: 18–19.
(29) SMITH, E. F.
1916. STUDIES ON THE CROWNGALL OF PLANTS. ITS RELATION TO HUMAN CANCER. Jour. Cancer Research 1: 231–258, illus.
(30) ———
1917. EMBRYOMAS IN PLANTS. (PRODUCED BY BACTERIAL INOCULATIONS). Bull. Johns Hopkins Hosp. 28 (319): [277]–294, illus.
(31) ———
1921. EFFECT OF CROWNGALL INOCULATIONS ON BRYOPHYLLUM. Jour. Agr. Research 21: 593–598, illus.
(32) ———
1922. APPOSITIONAL GROWTH IN CROWN-GALL TUMORS AND IN CANCERS. Jour. Cancer Research 7: 1–49, illus.
(33) ——— BROWN, N. A., and TOWNSEND, C. O.
1911. CROWN-GALL OF PLANTS: ITS CAUSE AND REMEDY. U. S. Bur. Plant Indus. Bull. 213, 215 pp., illus.
(34) SNOW, R.
1931. EXPERIMENTS ON GROWTH AND INHIBITION. I.—THE INCREASE OF INHIBITION WITH DISTANCE. Roy. Soc. [London], Proc. (B) 108: 209–223, illus.

(35) SNOW, R.
1931. EXPERIMENTS ON GROWTH AND INHIBITION. PART II.—NEW PHENOMENA OF INHIBITION. Roy. Soc. [London], Proc. (B) 108: 305–316.
(36) ———
1935. ACTIVATION OF CAMBIAL GROWTH BY PURE HORMONES. New Phytol. 34: 347–360, illus.
(37) ——— and LE FANU, B.
1935. ACTIVATION OF CAMBIAL GROWTH. Nature [London] 135: 149.
(38) THIMANN, K. V.
1935. ON THE PLANT GROWTH HORMONE PRODUCED BY RHIZOPUS SUINUS. Jour. Biol. Chem. 109: 279–291, illus.
(39) ———
1936. AUXINS AND THE INHIBITION OF PLANT GROWTH. (Abstract) Amer. Jour. Bot. 23 (Sup.): 689–690.
(40) ——— and KOEPFLI, J. B.
1935. IDENTITY OF GROWTH-PROMOTING AND ROOT-FORMING SUBSTANCES OF PLANTS. Nature [London] 135: 101–102.
(41) ——— and SKOOG, F.
1933. STUDIES ON THE GROWTH HORMONE OF PLANTS. III. THE INHIBITING ACTION OF THE GROWTH SUBSTANCE ON BUD DEVELOPMENT. Natl. Acad. Sci. Proc. 19: 714–716.
(42) ——— and SKOOG, F.
1934. ON THE INHIBITION OF BUD DEVELOPMENT AND OTHER FUNCTIONS OF GROWTH SUBSTANCE IN VICIA FABA. Roy. Soc. [London], Proc. (B) 114: 317–339, illus.
(43) ——— and WENT, F. W.
1934. ON THE CHEMICAL NATURE OF THE ROOTFORMING HORMONE. R. Acad. Sci. Amsterdam, Proc., Sec. Sci. 37: [456]–459.
(43a) WENT, F. W.
1928. WUCHSSTOFF UND WACHSTUM. Rec. Trav. Bot. Néerl. 25: 1–116, illus.
(44) ZIMMERMAN, P. W., CROCKER, W., and HITCHCOCK, A. E.
1933. INITIATION AND STIMULATION OF ROOTS FROM EXPOSURE OF PLANTS TO CARBON MONOXIDE GAS. Contrib. Boyce Thompson Inst. Plant Research 5: 1–17, illus.
(45) ——— CROCKER, W., and HITCHCOCK, A. E.
1933. THE EFFECT OF CARBON MONOXIDE ON PLANTS. Contrib. Boyce Thompson Inst. Plant Research 5: 195–211, illus.
(46) ——— and HITCHCOCK, A. E.
1933. INITIATION AND STIMULATION OF ADVENTITIOUS ROOTS CAUSED BY UNSATURATED HYDROCARBON GASES. Contrib. Boyce Thompson Inst. Plant Research 5: 351–369, illus.
(47) ——— and WILCOXON, F.
1935. SEVERAL CHEMICAL SUBSTANCES WHICH CAUSE INITIATION OF ROOTS AND OTHER RESPONSES IN PLANTS. Contrib. Boyce Thompson Inst. Plant Research 7: 209–229, illus.

A PHOTOELECTRIC SORTER FOR LENGTH MEASUREMENTS OF FIBERS OF SEED COTTON[1]

By BURT JOHNSON

Assistant agronomist, Arkansas Agricultural Experiment Station

INTRODUCTION

Both the cotton breeder and the textile engineer are concerned with the important factor of the length of cotton fibers, especially the average length of the fibers and the dispersion of lengths from the average. The original degree of uniformity of the fibers as found on the cotton seed may be disturbed by the effects of ginning, baling, compressing, and the several spinning operations. Although the breeder must not lose sight of the effects of the several industrial processes on fiber-length uniformity, he is more directly concerned with uniformity of the fibers as found on the cotton seed.

The purpose of this paper is to describe an apparatus for sorting cotton fibers as to length by the use of a photoelectric cell.[2] At present the method has not been adapted to ginned lint, or to multiple-seed sortings, but this fundamental or basic design of the apparatus can be adapted, if necessary, to fit other needs and problems. For example, modifications of this fundamental design to handle several seeds at a time might be made. As now constructed, the machine is especially applicable to the work of the cotton breeder and agronomist. It offers a rapid and practical means of sorting fibers as to length within satisfactory limits of accuracy.

METHODS OF OTHER WORKERS

Regardless of the contributing factors affecting the degree of uniformity of lint, there remains the very practical consideration of methods of measuring fiber lengths. Cotton breeders are especially anxious to use rapid means of sorting cotton fibers as to length, and mechanical sorters have been used to good advantage in breeding work. Several ingenious means of meeting this need along with discussions and criticisms of their utility may be found in the literature (*1*, *4*, *6*, *7*, *8*, *11*, *13*).[3] The purpose here is not to describe in detail each of these methods, but rather to discuss briefly certain mechanical or sampling problems as recorded by the authors of the methods or by critics of the methods.

The difficulty with most of the methods in present use, for practical purposes, is their slowness of operation. In some cases a considerable degree of skill must be developed before reliable results can be obtained. With all methods there is the difficulty of securing suitable

[1] Received for publication January 27, 1938; issued July 1, 1938. Contribution from the Department of Agronomy, Arkansas Agricultural Experiment Station. Research paper No. 498, Journal Series, University of Arkansas.

[2] The application of this particular technique to the sorting of cotton fibers originated with Dr. O. A. Pope while with the Arkansas Agricultural Experiment Station. The basic design of the machine which employed has been retained. The writer applied methods of maintaining steady light sources and used the apparatus extensively in breeding, genetic, and agronomic experimental work.

[3] Italic numbers in parentheses refer to Literature Cited, p. 56.

samples. Difficulty in sampling is not necessarily due to defects in the methods of sorting, but is inherent in the nature of cotton itself. Problems of sampling are met whether the sorting method uses the composite sample offered by ginned cotton, or samples taken from seed cotton. These sampling difficulties in the cotton itself may be partly overcome by more rapid sorting of larger samples. However, it has been pointed out that most of the variability found in lint of Pima cotton is contributed by the seed itself and larger samples of seed cotton would closely approximate the variability of lint lengths found on a single seed (*10*). This observation is probably applicable in somewhat less degree to other cottons.

A present method for ginned cotton as embodied in the Suter-Webb (*13*) and similar sorters has the advantage of a composite sample, but the process is slow. Any other method, such as Ball's sledge sorter, for ginned cotton also has the advantages of a composite sample if the sample is properly prepared. Ahmad and Nanjundayya (*1*) have recently described a sorter which determines the average length of ginned cotton perhaps more rapidly than other manual methods. However, on the face of it, it seems that this method would give only one statistic—average length—and would ignore the percentage of waste fibers, the model length, and dispersion from the average. By this method these authors also claim to obtain a measure of fineness.

Several methods for sorting lint still attached to the cotton seed have been devised in this country. Machines have been made to handle one (*6*) or more (*8*) seeds at a time. Such sorters have been used advantageously by breeders and agronomists (*9*), (*12*). The aim of these devices is to increase the speed of sorting and to take advantage of the approximate alignment of the basal ends of the fibers on the seed coat. McNamara and Stutts (*6*) have discussed the accuracy of this alignment and also the degree of alignment obtained at the distal ends of the fibers where they are grasped by the forceps.

All the methods thus far mentioned may under certain conditions give additional short fibers due to breakage during the sorting process itself. However, in the hands of a skilled operator, such possible breakage is probably a negligible factor.

Hertel and Zervigon (*4*) have employed a method of sorting fibers as to length by the use of a device employing photoelectric cells. This apparatus was originally designed to sort fibers on a single cotton seed. The advantage of the approximate alignment of the fibers on the seed coat was largely counteracted by the number of seeds required for a good sample. Nevertheless, this method for a single seed is comparatively rapid. This device is now used for sorting lint as found in rovings, and a theoretical consideration (*3*) has been given to the accuracy that can be obtained with ginned cotton partially parallelized in the manufacture of the roving.

Hertel's apparatus employs two photoelectric cells balanced against each other in relation to a single source of light and connected in parallel with a galvanometer. A cam, shaped according to considerations of theory and experimentation, is moved between the light and one of the photoelectric cells at a rate proportionate to the number of fibers lying between the light and the other photoelectric cell. The drawing of the cotton lint between the light and the photoelectric cell moves a pen, while the movement of the cam moves a card at right

angles to the movement of the pen. The results of the fiber length distribution are recorded as a curve on the card. Hertel has discussed the statistics that may be derived from this curve (*3*).

DESCRIPTION OF PHOTOELECTRIC LINT-SORTING APPARATUS FOR SEED COTTON

An apparatus somewhat different in principle from Hertel's has been used in the cotton fiber laboratory of the Arkansas Agricultural Experiment Station. The apparatus consists essentially of a boxlike cabinet having a curved slit in the lid. Suitable devices are provided for holding a combed cotton seed and for drawing the parallelized fibers across the slit. Inside the box and directly under the slit is mounted a photoelectric cell. This very simple arrangement is sup-

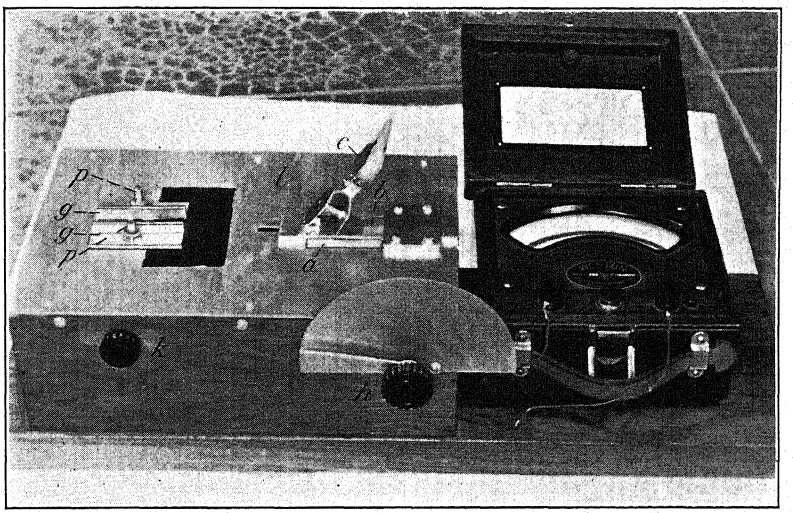


FIGURE 1.—View of assembled photoelectric cotton-sorting apparatus: *l*, Lid; *c*, clamp; *b*, bar; *h*, handle of clamp-moving device; *a*, scale; *k*, handle of guide-moving device; *p*, pillar; *g*, guide.

plemented by several accessories, as follows: To the photoelectric cell is connected a microammeter of 300 microamperes range. Light is directed downward on that part of the lint lying over the slit. In the light housing is a 6-volt, 18-ampere, ribbon-filament bulb. This shape of filament more adequately covers the long narrow slit than would other kinds of filaments. Through openings in the light housing, the light beam is passed through a series of lenses and mirrors (this type bulb must stand upright, base down) to place an image of the ribbon filament on the cotton over the slit. The current to the light bulb is passed successively through a voltage regulator for producing a steady current, a 6-volt transformer, and a common tumbler switch. A steady voltage is essential, but other methods for the same purpose might be used. The source of light is so arranged that two photoelectric sorters can be operated at the same time from the one light.

Figures 1 and 2 show in detail the construction of the sorting apparatus. The slit (*s*) in the lid (*l*) is curved slightly as a partial compensation for the curvature of the seed. The inside and the outside

arcs of the slit are made on radii of three fourths and thirteen sixteenths of an inch, respectively, and are concentric. The distance on a straight line through this slit varies from place to place. Therefore, a slit made so that the edges are eccentric to each other might produce a more accurate reading. Guides (*g*) can be opened or closed over the slit by turning a knob (*k*). The guides for all the work reported in this paper were seventeen thirty-seconds of an inch apart. The pillars (*p*), to which the guides are fastened, are moved backward and forward by left- and right-hand threads on the shaft (*m*). The purpose of the guides is to confine all the lint over the slit. By adjusting the space between them, the width of opening for any par-

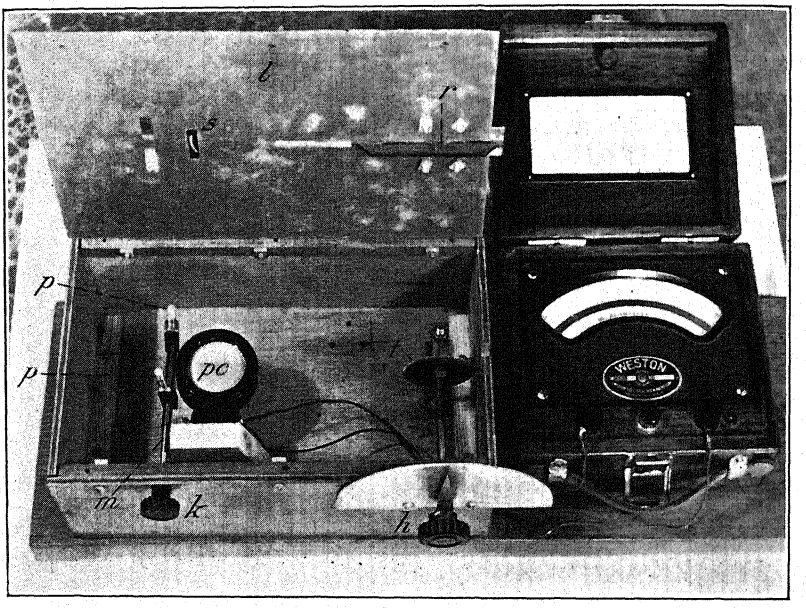


FIGURE 2.—View of photoelectric cotton sorting apparatus with lid of cabinet laid back to show interior of cabinet: *s*, Slit; *r*, rack; *t*, gear; *pc*, photoelectric cell; *m*, shaft with right- and left-hand threads. Other symbols as in figure 1. The microammeter at right is connected directly to the photoelectric cell.

ticular requirements of light or sample can be met. The photoelectric cell is indicated by *pc*.

A previously combed cotton seed is placed in the clamp (*c*). The gear (*t*), moved by turning (*h*), engages the rack (*r*). This rack is attached to the bar (*b*), to which the clamp (*c*) is fastened. On the bar is a mark which is alined with the zero of the scale (*a*), having 1/16-inch divisions.

In operation, the fibers, after being combed, are further straightened as much as possible between the fingers, and the combed seed is then placed in the clamp. The clamp is swung downward and the fibers are placed between the guides. By manipulation with the fingers, the fibers are straightened out as much as possible between the guides, and the mark on the bar brought up to the 1/16-inch mark on the scale. A reading is made from the microammeter. The bar, clamp, and seed are moved back one sixteenth-inch more, and another reading from

the microammeter is recorded. This is repeated until all the fibers have been pulled past the slit, a reading being taken each one-sixteenth inch. The final reading is a blank with no fibers over the slit.

The seed is then removed from the clamp, reversed, the fibers on the other half of it straightened, and the combed seed returned to the clamp ready to have the second half of the lint sorted. In this way all the fibers are sorted, eliminating the very serious error that may occur when only part of the fibers is considered. The great irregularity in lint length that may occur on a single seed has been noted by other investigators, among whom may be mentioned Cook (*2*), Hertel and Zervigon (*4*), Pope (*7*), and Richmond and Fulton (*10*). Class lengths other than one-sixteenth of an inch may well be used when the degree of separation need not be too minute. For most practical or routine purposes the ⅛-inch class length is perhaps to be preferred. In practice it has been found that when using

Length in ⅛ inch (L)	Readings in μ amps		(2) plus (3)	New and adjusted readings in μ amps	Absorption in μ amps	Percent absorption	Fractional number of fibers in percent (F)	Cumulative percentage	LF
	First half	Second half							
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
1	70	34	104	0	372	100.00	0.00		
2	73	34	107	3	369	99.19	.81		1.62
3	75	35	110	6	366	98.39	.80		2.40
4	75	38	113	9	363	97.58	.81	2.42	3.24
5	83	42	125	21	351	94.35	3.23		16.15
6	102	65	167	63	309	83.06	11.29		67.74
7	157	124	281	177	195	52.42	30.64		214.48
8	227	194	421	317	55	14.78	37.64		301.12
9	238	238	476	372	0	0.00	14.78	100.00	133.02
10									

Percent ½ inch and less	::Percent longer than ½ inch	
Modal length		
Percent number of fibers in three 1/8-inch primary modal groups		
Σ of (10) 739.77	Mean 7.40/8 inch - ± /8 inch	Coefficient of variability

FIGURE 3.—Form of work sheet and sample calculation for the length sorting of fibers from one cotton seed.

the ⅛-inch class length, an operator can sort an average of 12 seeds an hour. This average is based on the use of long-, medium-, and short-staple cottons just as they come to hand from a randomized variety test. The seeds are previously combed, and this operation would reduce the number of sortings per hour if the operator also had to comb his cotton.

CALCULATIONS

The present method of calculating the data from the work sheets is as follows: The readings at the corresponding length groups of both halves (columns 2 and 3, fig. 3) of the combed seed are added (column 4). This includes the final or blank reading. Next the total reading from the shortest length group measured (one-eighth inch) is then subtracted in turn from each of the other readings, giving readings based on the relative number of fibers found in that particular sample, as shown in column 5. As a consequence, the first reading is always zero. Then, these new readings are each in turn subtracted from the combined blank (or final) reading, which gives the amount of light absorbed by the fibers (column 6). The

percentage absorption is found by dividing the blank (or final) reading into each of the absorptions at the several length intervals in turn. These percentages are recorded in column 7. As a result, the first reading will be 100 percent absorption and the final reading no absorption.

The determination of the relative number of fibers in a length group is accomplished by subtracting the percentage absorption for that length group from the next shortest length group, or simply by subtracting the percentage of absorption from the one above it (column 8). In the data given in this paper, the bottom point of each length group has been used as the point of measurement. The cumulation of the fractional length percentages (column 9) not only serves to show the percentages of fibers in a set of length groups (e. g. the percentage one-half inch and less in length), but it also acts as a partial check on the accuracy of the subtractions in the "percentage of absorption" column. Or an ogive curve could be constructed directly from the cumulations if each were entered in column 9 (such data are not shown in fig. 3).

The proportionate total length of fibers in any length group is found by multiplying the value of each length group (column 1) by the percentage fractional number of fibers for the corresponding length group (column 8). The result is placed in column 10. These values are then added and the sum divided by 100 to give the mean length of fibers in the entire sample. In the illustration (fig. 3) the class length used was one-eighth inch.

From data of this type, the modal group is readily selected, percentages of fibers in any one length group or in combinations of length groups are readily obtained, mean length based on relative number of fibers is determined, and measures of dispersion may be calculated. Figure 3 shows some of these data with suggestions as to other possible statistics.

In practice, a table is used to convert the readings in column 5 to "absorption in microamperes" and "percent absorption" (columns 6 and 7, respectively). As a further saving of time, the data in column 6 usually are not copied. The data in this column, after having been used in the calculation of "percent absorption" in the conversion table, do not enter further into the computations on the work sheet.

EXPERIMENTS WITH THE PHOTOELECTRIC SORTER

A large number of samples of seed cotton from various kinds of fertilizer, breeding, and genetics tests have been sorted with the photoelectric sorter. These data are not presented here in toto, but only such parts as show the advantages, disadvantages, and degree of accuracy of the method. The photoelectric sorting method gives satisfactorily comparable results in a series of determinations. Such measurements would be of comparable value among themselves, but might be difficult to correlate in terms of other methods. A method may also be compared directly to some method whose accuracy is generally accepted as giving a true concept of the reliability of the method under test. Both kinds of data are given below.

It is necessary to know whether or not repetition of sortings on a given sample by the photoelectric sorting method will give duplicate results. Data from an experiment to establish whether or not results

secured by operator A using instrument A could be repeated by operator B with instrument B are found in tables 1 and 2. No attempt had been made to calibrate these sorters in terms of one or the other. However, the effect of the intensity of light on results will later be pointed out. In this experiment 10 cotton seeds were selected representing various lengths of staple, each seed being an individual sample. Operator A made three photoelectric fiber sortings of each seed on instrument A. Then operator B, using instrument B, repeated this process three times with the same samples. By using Student's method (as discussed by Love and Brunson (*5*)), the odds that a real difference in results in this experiment were found to be 38:1. The raw data are given in table 1. The average lengths found for each sorting were determined separately and recorded in the columns "mean lint lengths of individual sortings" of table 1. The extreme differences of the mean lengths and the mean of three lint sortings of each seed are likewise recorded.

TABLE 1.—*Comparison in detail of average length of cotton lint from 10 separate seeds fractioned by 2 operators on 2 photoelectric sorters*

[Each operator made 3 sortings of lint on each seed. A sample consists of 1 seed]

Sample No.	Instrument A, operator A			Instrument B, operator B		
	Mean lint lengths of individual sortings	Extreme difference of mean lengths	Mean of 3 lint sortings of each seed	Mean lint lengths of individual sortings	Extreme difference of mean lengths	Mean of 3 lint sortings of each seed
	1/16 inch	*1/16 inch*	*1/16 inch*	*1/16 inch*	*1/16 inch*	*1/16 inch*
1	12.96 12.95 12.96	0.01 (0.000625 inch)	12.96	13.95 14.21 14.16	0.26 (0.01625 inch)	[1] 14.11
2	13.76 13.79 13.80	.04 (.0025 inch)	13.78	15.06 14.57 14.99	.49 (.030625 inch)	[2] 14.87
3	16.09 16.24 16.20	.15 (.009375 inch)	16.18	16.67 16.28 16.43	.39 (.024375 inch)	16.46
4	15.42 15.54 15.39	.15 (.009375 inch)	15.45	15.19 15.18 15.22	.04 (.0025 inch)	15.20
5	11.88 11.94 11.91	.06 (.00375 inch)	11.91	11.72 11.74 11.87	.15 (.009375 inch)	11.78
6	12.21 12.21 12.21	.00	12.21	11.98 12.30 12.31	.33 (.020625 inch)	12.20
7	19.12 19.22 19.40	.28 (.0175 inch)	19.25	19.53 19.58 19.62	.09 (.005625 inch)	19.58
8	18.12 17.80 18.17	.68 (.0425 inch)	[3] 18.03	18.96 19.04 19.21	.25 (.015625 inch)	19.07
9	19.22 19.07 19.17	.15 (.009375 inch)	19.15	19.43 19.82 19.62	.39 (.024375 inch)	19.62
10	18.44 18.45 18.45	.01 (.000625 inch)	18.45	18.72 18.93 19.04	.32 (.02 inch)	18.90

[1] Operator B inexperienced with this instrument. Sample resorted by A, who found an average length of 13.42 sixteenths of an inch.
[2] Operator B inexperienced with this instrument. Sample resorted by A, who found an average length of 13.90 sixteenths of an inch.
[3] Careless sorting by A. Resorted by A, who found an average length of 18.23 sixteenths of an inch.

In table 1, attention is directed to the wide differences between the results on the two sorters in the case of samples 1, 2, and 8. The fact that the other differences are so slight would indicate as regards

these particular samples that one or both of the operators were in error. For samples 1 and 2, operator A was inexperienced in the use of instrument B, and a more nearly correct sorting by operator B was made. Apparently, with sample 8, both operators were in error, especially B. But the error is so obvious that a redetermination was indicated. This was done, with the results shown. It seems that an acceptable degree of agreement between two operators using two instruments not especially calibrated in terms of a standard can be expected. Further, it seems that a single operator can be expected to produce consistent results, his extreme difference in the determination of mean length of lint on a seed seldom being much over 0.02 inch.

TABLE 2.—*Comparison in detail of modal lengths of cotton lint from 10 separate seeds fractioned by 2 operators on 2 photoelectric sorters*

[Each operator made 3 sortings of lint on each seed. A sample consists of 1 seed]

Sample No.	Instrument A, operator A		Instrument B, operator B		Extreme difference of all modes by both sorters	Sample No.	Instrument A, operator A		Instrument B, operator B		Extreme difference of all modes by both sorters
	Modes of 3 trials	Extreme difference of modes	Modes of 3 trials	Extreme difference of modes			Modes of 3 trials	Extreme difference of modes	Modes of 3 trials	Extreme difference of modes	
	1/16 *inch*	1/16 *inch*	1/16 *inch*	1/16 *inch*	1/16 *inch*		1/16 *inch*	1/16 *inch*	1/16 *inch*	1/16 *inch*	1/16 *inch*
1	14	0	14	1	1	6	12	0	12	1	1
	14		14				12		13		
	14		15				12		13		
2	13	1	14	0	1	7	20	1	20	0	1
	14		14				19		20		
	14		14				19		20		
3	17	0	17	0	0	8	19	0	19	1	1
	17		17				19		19		
	17		17				19		19		
4	16	1	16	1	2	9	19	1	18	1	
	17		16				19		19		
	17		15				20		19		
5	12	0	12	1	1	10	19	0	19	0	0
	12		12				19		19		
	12		13				19		19		

Equally consistent results from the same experiment are shown in the determination of the modal lengths of the lint from the 10 seeds (table 2). These determinations were made at 1/16-inch intervals. In routine work 1/8-inch intervals are generally used. Such being the case, the modal lengths in this experiment probably would have agreed even more closely had the 1/8-inch length grouping been used with its consequent sacrifice of the accuracy of the finer readings.

Ninety other seeds were sorted in a manner similar to the one described for the 10 seeds referred to above, except that each operator made only one sorting to a seed instead of three. The results are collected in table 3, where the differences between the two operators and two instruments, as shown by comparisons in mean length and modal length of lint found on a given seed, are combined in group values of tenths of 1/16 inch, and the number of averages showing the corresponding range of differences are placed in the designated columns. In comparing the differences in modal lengths a class value of 1/16 inch (the interval at which measurements were made) is used. Again, it will be seen that the agreement continues to be close, most of the averages showing a difference of less than 1/32 inch, and the

modal lengths usually being equal or separated by not more than one length class.

Mention has been made of the importance of the intensity of light. Reference to table 1 will show that 7 times out of 10 a higher reading was secured on instrument B than on A. This might have been due to an error on the part of the operator rather than to an error in the method. The photoelectric cell in B has about one-half the amperage output of the cell in instrument A. Therefore, the light intensity on the cell in B, other things being equal, will have to be considerably greater than that on the cell in A, if the amperage output is to be equal.

TABLE 3.—*Grouped comparisons of average and modal lengths of lint on 90 cotton seeds sorted on 2 photoelectric fiber sorters by 2 operators*

[Class differences for mean lengths are in terms of tenths of a sixteenth of an inch; class differences for modal lengths are in terms of one-sixteenth of an inch]

Difference in mean lengths (1/16 inch)	Frequency	Difference in modal lengths (1/16 inch)	Frequency
0.00 to 0.10	16	0.00	50
0.11 to 0.20	18	1.00	37
0.21 to 0.30	12	2.00	3
0.31 to 0.40	17		
0.41 to 0.50	8		
0.51 to 0.60	9		
0.61 to 0.70	3		
0.71 to 0.80	2		
0.81 to 0.90	2		
0.91 and above	3		
Total	90	Total	90

In a test in which the current outputs from both instruments were adjusted to be approximately equal, the following averages on the same sample were made by one operator:

Instrument A.—7.16; 7.12; 7.12; 7.02; 7.07. Extreme difference, 0.14-eighths of an inch.

Instrument B.—7.49; 7.46; 7.50. Extreme difference, 0.04-eighths of an inch.

For instrument B, the modal length was eight-eighths of an inch and that for A, seven-eighths of an inch.

As seen from the data, there are consistently higher readings from the sorter with the weaker cell. This may be due to the relationship between the quality of light of high intensity to absorption by lint as it affects a photoelectric cell. However, the writer is of the opinion that most of the discrepancy in this case is due to the comparatively small area of the light image on the fibers. In order to obtain a high amperage output from instrument B, it was necessary to have an intense beam of light in which the outer fringes of the light image are definitely less intense than toward the center. The shorter fibers often found toward the ends of the seed are not as well scanned in the much weaker light of this outer fringe. If these short fibers were incorporated more accurately in the sortings done on instrument B, very likely the readings would be more nearly comparable to those on A. Instrument A can show a high reading on the microammeter and still have a band of light of even intensity extending far beyond the mass of fibers being scanned. The later practice in this laboratory has been to sacrifice, in the case of instrument B, the accuracy of reading

expected with a high current output for the greater accuracy of a more diffuse light more equally scanning all fibers. In spite of this, exact comparability has not yet been achieved. It appears that apparatus of this sort should be equipped with the more sensitive type of cells and all instruments formally calibrated against some standard instrument.

When data from the same 90 seeds referred to in table 3 were used, the total of the mean lengths as given by instrument A was 1.91 percent shorter than the total of the mean lengths given by instrument B on the same samples. This is of the same general magnitude as the differences shown in table 1, and a similar figure might be used as a correction factor in order to get these sorters calibrated in terms of one of them.

Ten samples of seed cotton, some samples consisting of one seed only, and the others of two seeds, were sorted by the photoelectric method. The fibers from each seed were then carefully ginned by hand, and in the case of the samples with two seeds, the fibers from both seeds were combined into a composite sample. An aliquot of each sample of ginned lint was withdrawn and the individual fibers measured to the nearest one-sixteenth of an inch. The results are given in table 4. In all cases the measurements of fibers less than five-sixteenths of an inch long were disregarded, because, except in the shortest staple cottons, the photoelectric method does not accurately measure the short fibers. This point will be discussed below.

In this experiment the agreement between the photoelectric method and measurements of single fibers is fairly close in the case of the short and medium staples, considering the limitations of such an experiment in the factors of the size of aliquot used and the difficulty of obtaining a representative aliquot. The sources of error in the photoelectric method seem to be largely inaccurate measurement of the few shortest and longest fibers.

TABLE 4.—*Comparison of mean and modal lengths as found by photoelectric sorting with measurements of individual fibers*

Sample No.	Seed No.	Single fibers measured	Mean length by—		Modal length by—	
			Photoelectric sorting	Single-fiber measurements	Photoelectric sorting	Single-fiber measurements
		Number	*1/16 inch*	*1/16 inch*	*1/16 inch*	*1/16 inch*
1	1	937	15.09	15.24	16	15
2	1	1,141	15.55	15.84	16	17
	2		16.53		17	
3	1	1,223	19.22	20.72	20	23
	2		18.17		19	
4	1	554	18.56	18.22	19	20
5	1	438	19.93	18.20	21	22
6	1	789	14.44	14.04	15	15
7	1	590	11.00	11.34	11	11
8	1	620	13.40	13.42	13	14
9	1	1,252	10.58	10.79	11	11
	2		9.51		10	
10	1	475	15.88	14.81	16	15

In table 5 are data on the number of seeds required for accuracy in photoelectric sorting. As will be noted, no attempt was made to remove the effect of locations by variance. Thus this test was very

rigorous and the number of seed required, as shown, is probably more than sufficient for most purposes for the accuracy indicated. Each of the 5 varieties was grown at 12 locations, scattered to give a wide range of the soil and climatic types found in the cotton-growing districts of Arkansas. The 6 locations (superior locations), where the lint was longest as shown by stapling, furnished 92 seeds sorted for each variety. The other 6 locations (inferior locations) were in the dry and hill sections of the State and furnished 96 seeds sorted for each variety. Nevertheless, the variability from location to location is marked even within the groups of "inferior locations" or "superior locations." To add to the rigor of the test, both instruments and two operators sorted these samples, and the cotton from one of the locations was sorted by three different operators.

TABLE 5.—*Size of sample required for specified accuracies in determination of mean length of fibers by means of photoelectric sorting*

[See text for source of populations]

Variety	Seeds tested	σ	Number of seeds (n) for an accuracy of—	
			$\frac{2\sigma}{\sqrt{n-1}}$ = 1/32 inch	$\frac{2\sigma}{\sqrt{n-1}}$ = 1/16 inch
	Number	*1/8 inch*	*Number*	*Number*
INFERIOR LOCATIONS				
Coker-Wilds 7	96	0.583	23	7
Missdel 3-9818	96	.688	32	9
D. & P. L. 11A	96	.506	18	6
Rowden 2088	96	.550	21	6
Half and Half	96	.462	15	5
SUPERIOR LOCATIONS				
Coker-Wilds 7	92	0.637	25	8
Missdel 3-9818	92	.696	32	9
D. & P. L. 11A	92	.682	31	9
Rowden 2088	92	.545	20	6
Half and Half	92	.543	20	6

In sorting 216 seeds of Rowden 5056, taken from a fertilizer test at one location, a standard deviation of 0.52 eighth of an inch was found for the entire test. This sorting was done by three comparatively inexperienced operators. The sizes of samples required were:

Where $n=40$ seeds, $\frac{3\sigma_x}{\sqrt{n-1}}=1/32$ inch

Where $n=11$ seeds, $\frac{3\sigma_x}{\sqrt{n-1}}=1/16$ inch

Where $n=19$ seeds, $\frac{2\sigma_x}{\sqrt{n-1}}=1/32$ inch

Where $n=6$ seeds, $\frac{2\sigma_x}{\sqrt{n-1}}=1/16$ inch

Variance was applied to data from 16 varieties in the 12 locations mentioned above, as regards the factors of mean length and modal length. The plantings in these tests were so arranged that this method of statistical analysis was applicable. Eleven of these tests were 16 × 8 semi-Latin squares and one was a 24 × 6 semi-Latin square. They were all randomized and doubly restricted. A summary of the standard errors of the individual is given in table 6. These standard errors are somewhat lower than those shown in table 5, because the variance removes the variation due to varieties and to soil variations in both directions across the field. The regularity of these standard errors and the similarity of the differences required for significance between varieties all tend to show the uniformity of work done by the photoelectric method.

TABLE 6.—*The variance of 16 varieties of cotton at 12 locations in Arkansas, showing a comparison between locations as regards the standard error of the individual, and the significant differences between varieties in the factors of mean length and modal length of lint*

Location	Standard error of individual		Difference required for significance between varieties			
			Mean		Mode	
	Mean	Mode	19:1	99:1	19:1	99:1
	1/8 inch	*1/8 inch*	*1/8 inch*	*1/8 inch*	*1/8 inch*	*1/8 inch*
Warren	0.5319	0.5083	0.52	0.69	0.50	0.66
England	.4022	.4515	.39	.52	.44	.58
Marianna	.3871	.3876	.38	.50	.38	.50
Hope	.3900	.4261	.44	.58	.48	.63
Magnolia	.3845	.4575	.38	.50	.45	.59
Lake Village	.4419	.4538	.43	.57	.44	.59
Joiner	.3822	.4061	.37	.49	.40	.52
Blytheville	.3743	.4140	.37	.48	.41	.53
Brookland	.3809	.4281	.37	.49	.42	.55
Myron	.3859	.4425	.38	.50	.43	.57
Damascus	.4787	.5341	.47	.62	.52	.69
Newport	.4011	.3921	.39	.52	.38	.51

The results from the tests at Warren and Damascus (table 6) require further explanation. The standard errors in both cases deviate noticeably from the general magnitude of the standard errors at the other locations. The test at Warren was sorted by three relatively inexperienced operators. The test cotton at Damascus was grown under conditions of such extreme drought that the lint was very immature, brash, difficult to manipulate, and perhaps quite irregular. The photoelectric method seems to be sensitive enough to detect these irregularities induced by inexperienced operators or conditions under which the crop is grown.

From all the evidence it seems that sorting a sample of 10 seeds from each row in each location by the photoelectric method would be amply sufficient to give an accurate result. If this number had been used in the work reported in table 5, a total of 480 seeds would have been sorted for each variety for the inferior locations and 460 for the superior locations. The degree of precision provided by a 10-seed sample is probably great enough for most studies on utility and genetics. Hertel and Zervigon (*4*) say that at least six seeds are required in the sample for their photoelectric sorter.

The error arising from the inability of the photoelectric sorter adequately to scan the shortest fibers is one of the most difficult to explain and to rectify. The seriousness of this error is shown in table 7. These results were secured by making a photoelectric sorting of one-half of the fibers of each of six seeds. These fibers were then sorted on the McNamara-Stutts sorter. All the fibers lying between any two combs of the sorter were grouped into a class interval of one-eighth inch. The readings for the McNamara-Stutts sorting as recorded in the table are conservative. In column *P* is given the length class which showed by photoelectric sorting a higher reading on the ammeter than the first reading for the ⅛-inch length class (i. e., it is the first length class showing fewer fibers than the shortest length class).

TABLE 7.—*Numbers of fibers not adequately recorded by the photoelectric fiber sorter*

[Column *P* records the length class where the first positive reading with the photoelectric sorter was made

Seed No.	Photoelectric sorter			McNamara-Stutts sorter		Fibers shorter than length in column *P*		Weight of fibers, in relation to total weight of all fibers, shorter than length in column *P*
	P	Mean	Mode	Mean	Mode			
	⅛ inch	*⅛ inch*	*⅛ inch*	*⅛ inch*	*⅛ inch*	*Number*	*Percent*	*Percent*
1	6	9.69	10	8.67	10	562	15.51	6.74
2	6	9.67	10	8.74	10	668	17.67	8.10
3	6	9.34	10	9.20	9	539	9.01	3.59
4	8	10.31	11	9.92	11	675	15.26	7.23
5	7	9.81	10	9.11	10	528	14.97	7.93
6	4	9.55	10	8.89	9	162	4.60	.65

The number of fibers of less length than the first positive reading in column *P* were counted. These do not include fuzz up to one-fourth inch long. As can be seen, this number is not small, and when put on a percentage basis, shows even more strikingly the importance of the neglected fibers. On the weight basis, the loss, although not so great, remains considerable. The results shown in the table are the extreme, and can be compared with the result shown in figure 3, where apparently a practically accurate reading of the shorter lint has been achieved. Most readings will not show the extreme differences shown by these readings on long-staple cotton, as recorded in table 7. For example, 10 seeds from a sample of Rowden 5056 averaged 357 fibers less than one-half inch long. These 10 included seeds that showed by photoelectric sorting either no fibers less than one-half inch long or no fibers less than three-eighth inch long. The number of fibers not shown by the sorting would be somewhat reduced if only the fibers shorter than the shortest shown by the photoelectric sorter had been counted. The purpose here is to show at its very worst a source of error in photoelectric cotton sorting as it is now performed. It is to be noted (table 7) that the modes as determined by the two methods practically coincide.

DISCUSSION

The method of calculation at present employed assumes that the profile of the fiber has no marked effect on the light absorption over

its entire length; that the graph of the logarithmic absorption of light caused by the addition of layers of absorptive material approaches a straight line; that the refraction of light probably compensates partially for the logarithmic absorption effect; and that all fibers in a given sample absorb light equally.

The fusiform shape of the fibers has been noted by several workers. Obviously there are more fibers at a point close to the seed coat than at a point farther away from the seed coat. However, in photoelectric sorting, in many cases, especially in samples with long staples, the reading at a point nearest the seed coat is greater (indicating less absorption) than at points immediately after the first reading. (See also table 7.) Hertel and Zervigon (*4*) have noted the same phenomenon. Weights of the ends of fibers are less than the middle portions of equal length of the same fibers (*1*), indicating not only a smaller diameter at the end of the fiber, but also a less absolute amount of absorptive material in the basal end of the fiber. Another reason for the initial low absorption is the shape of the clamp holding the cotton seed. The two prongs of the clamp pass between the fibers and separate them, permitting more light to pass through these openings between the more densely packed fibers. Also the fibers are not quite in the same alignment in relation to the slit next to the seed as they are farther out from the seed coat.

The profile of the fiber cannot readily be changed. It may be that after more experience a correction factor can be derived to correct for the fusiform shape of the fiber. It is likely that a similar correction factor would also have to be applied to the distal ends of the lint. The clamp might be redesigned. One possibility would be to have needle-sharp prongs that would penetrate the seed coat. An objection to this is that such prongs would make it even more difficult than it now is to aline the edge of the seed with the scale.

In theory, the fibers near the seed, being more numerous, should absorb more light than the fewer fibers extending farther from the seed coat. This is expressed ideally by: $\text{Log } \frac{I}{I_o} = -nx$, where I_o equals the original intensity of the light; I equals the intensity of the light after passing n quantity of material with each increment of the material having an absorptive capacity of x. In this equation the ideal is assumed that the absorptive material reflects none of the light, which is not the exact fact as regards cotton fibers.

The effect of reduced light by the absorption of light by added layers of lint is illustrated in the following experiment: Groups of lint, judged to contain about the same number of fibers, were placed on top of one another over the slit of the photoelectric sorting apparatus. After the addition of each group of fibers a reading was recorded. Then the differences between each successive reading were found to determine the absorption by that group of fibers. The differences between successive readings in microamperes were as follows: 12, 24, 15, 42, 21, 17, 19, 7, 19, 14, 4, 8, 3, 8, 5, 7, and 5. The first absorptions are of the same general magnitude, with later a definite trend toward smaller absorptions. There are variations in the above readings because of variations in the number of fibers added from group to group. In general, about 150 fibers were added each time, making

in all about 2,550, or the approximate number of fibers on one-half of a combed seed.

A variation of this experiment in which the light intensity is increased after a number of groups of fibers have been added will increase the absorption of other added groups, but the absorptions will later become lower.

Other possible causes of error are here mentioned. Because the slit through which the light passes is one-sixteenth inch wide, a correction might be subtracted from a reading to account for the various fiber lengths that would fall in the 1/16-inch interval. A possible further correction would be to correct for the size of the length interval of lint drawn past the slit each time. That is, the midpoint of the length class might be the measuring point instead of the bottom point as was used in all work reported in this paper. Thus far, no such corrections have been attempted. But the disagreement between the averages by photoelectric sorting and by measurement of individual fibers (or counting measured fibers) suggests that a correction for photoelectric readings be considered. The effect of changing the arcs bounding the slit from a concentric to an eccentric relationship, as well as other changes in the construction of the apparatus, were noted in describing the photoelectric sorter.

This problem of the accurate measurement of the shorter fibers is of importance in other methods of sorting. The tendency has been to consolidate into one class all fibers of less than a given length, say one-half or three-fourths inch (*6*, *10*). Pope (*7*) has discussed the advisability of omitting the short fibers from a consideration of the mean length and deviations from the mean. He points out that since most short fibers do not enter into the final spining product, the spinner is concerned mostly with the long fibers as they affect the quality of his product. But the cotton breeder is interested in producing as many long fibers in relation to the number of short fibers as possible. In order to determine this relationship properly, it would seem that a quick and accurate measurement of the short fibers is to be desired by the breeder, and that efforts to achieve such measurements should be encouraged. Then, if for certain purposes it is not necessary to have the short fibers sorted in detail, it is still possible to consolidate such readings as may be desired into one length class.

SUMMARY

A description of a basic design for a cotton-fiber sorter employing a photoelectric cell is given. Sorting fibers on seed cotton as to length by this method is comparatively rapid, but sources of error and possible means of improving the technique and the construction of the apparatus are pointed out.

The method of calculating the data is simple, readily providing statistics on average length, modal length, percentage of fibers in any length of group or groups, and data from which measures of dispersion may be obtained.

This apparatus has been applied to many sortings for use in making selections in breeding and genetics studies and in various cotton variety and fertilizer tests.

A close agreement was found between operators and instruments on the same samples, although the instruments have not been calibrated to

a standard. Ten cotton seeds should ordinarily provide an adequate sample for photoelectric sorting. There is a fairly close agreement between photoelectric sorting and measurements of single fibers, as regards average length and modal length.

The principal error in the photoelectric method of sorting fibers appears to be an inadequate consideration of the shortest fibers. The few very longest fibers also are not accurately measured. The effect of the structure of the fiber and the logarithmic absorption of light in relation to these errors are discussed.

LITERATURE CITED

(1) AHMAD, NAZIR, and NANJUNDAYYA, C.
1936. A NEW METHOD AND APPARATUS FOR DETERMINING THE AVERAGE LENGTH AND FINENESS OF COTTON HAIRS. Indian Cent. Cotton Com. Technol. Lab., Technol. Bull. Ser. B, No. 21, 22 pp., illus.

(2) COOK, O. F.
1931. INEQUALITY OF COTTON FIBERS. A DIRECT METHOD OF COMPARING SUBSTAPLE. Jour. Heredity 22: 25–34, illus.

(3) HERTEL, K. L.
1936. APPLICATION OF THE SEED COTTON FIBROGRAPH TO LINT COTTON. Jour. Tenn. Acad. Sci. 11: 147–152, illus.

(4) ——— and ZERVIGON, M. G.
1936. AN OPTICAL METHOD FOR THE LENGTH ANALYSIS OF COTTON FIBERS. Textile Research 6: 331–339, illus.

(5) LOVE, H. H., and BRUNSON, A. M.
1924. STUDENT'S METHOD FOR INTERPRETING PAIRED EXPERIMENTS. Jour. Amer. Soc. Agron. 16: 60–68.

(6) MCNAMARA, HOMER C., and STUTTS, ROBERT T.
1935. A DEVICE FOR SEPARATING DIFFERENT LENGTHS OF FIBERS FROM SEED COTTON. U. S. Dept. Agr. Cir. 360, 16 pp., illus.

(7) POPE, O. A.
1933. THE CALCULATION OF CERTAIN FIBER LENGTH CONSTANTS IN COTTON. Jour. Amer. Soc. Agron. 25: 740–756, illus.

(8) PRESSLEY, E. H.
1933. A NEW TYPE OF COTTON SORTER. Jour. Amer. Soc. Agron. 25: 89–98, illus.

(9) ———
1934. IMPROVING THE UNIFORMITY OF COTTON FIBER BY THE USE OF THE PRESSLEY SORTER. Ariz. Agr. Expt. Sta. Tech. Bull. 54, pp. 127–153, illus.

(10) RICHMOND, T. R., and FULTON, H. J.
1936. VARIABILITY OF FIBER LENGTH IN A RELATIVELY UNIFORM STRAIN OF COTTON. Jour. Agr. Research 53: 749–763, illus.

(11) SOKURAJA-VYSOTSKAJA, O.
1935. MEASUREMENTS OF THE LINT LENGTH OF COTTON UNDER FIELD CONDITIONS. AN APPARATUS CONSTRUCTED BY THE CENTRAL BREEDING STATION, N I H I. Bor'ba za Khlopok 8/9: 127–132, illus. [Original not seen. Abstract in Empire Cotton Growing Rev. 13: 233. 1936.]

(12) STROMAN, G. N.
1934. COTTON BREEDING INVESTIGATIONS, 1928 TO 1932. N. Mex. Agr. Expt. Sta. Bull. 217, 39 pp., illus.

(13) WEBB, R. W.
1932. THE SUTER-WEBB COTTON FIBER DUPLEX SORTER AND THE RESULTING METHOD OF LENGTH-VARIABILITY MEASUREMENTS. Amer. Soc. Testing Materials Proc. 35, v. 32, pt. 2, pp. 1–11, illus.

VARIABILITY IN GERMINATION OF FRESHLY HARVESTED AVENA [1]

By F. A. COFFMAN, *agronomist*, and T. R. STANTON, *senior agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Freshly harvested seeds often do not germinate satisfactorily. From some points of view this slowness to germinate is undesirable, but the possibility that it may have certain advantages should not be overlooked. Growers have long been cognizant of the heavy losses resulting from the sprouting of grain in the bundle or shock. Obviously, from the practical viewpoint, delayed germination might prove extremely desirable in a wet harvest season. Any variety in which germination is retarded even for a few days, when moisture and temperature conditions are favorable, might escape considerable damage in the field. Comparatively little attention has been given as yet to the few reports of the wide variation in the germinability of freshly harvested seed of different varieties of the small grains. It seemed important, therefore, to determine the extent of such variation among varieties of oats.

REVIEW OF LITERATURE

Sifton (*13, p. 23*) [2] stated that "in the majority of cases there is a slight rise in the germination of oats during the first 4 or 5 years of storage."

Harrington (*7*) cites Kinzel (*10, p. 133*) as saying that oats reached their full germination capacity in 2 months after harvest, but their full germination energy only after 8 or 10 months.

Whitcomb (*15, 16*) stated: "The curing in the field had a slight beneficial effect on the initial germination but it was not well defined" (*15, p. 87*), and "Aging grain in the laboratory for from 4 to 12 weeks decreased the dormancy to a minimum" (*16, p. 32*).

Garber and Quisenberry (*6*) found that in crosses between *Avena sativa* L. and *A. fatua* L. delayed germination is an inherited recessive character and that it is somewhat loosely linked with the *fatua* type of seed articulation. They apparently recognized a variation in degree of dormancy among different strains of *A. fatua* in their cultures.

Eastham (*3, p. 81*) stated:

> In wet, cold harvest years many samples of Black Winter oats do not germinate readily under normal laboratory conditions. Although such Oats will grow perfectly well under field conditions, yet they may only show a germination of from 20 to 30 per cent. after several weeks in test.

Eastham pointed out that merely lowering the temperature of the germination chamber is helpful, or if this proves unsuccessful, the placing of the samples on ice for 4 or 5 days will readily overcome the difficulty.

[1] Received for publication December 22, 1937; issued July 1, 1938.

[2] Italic numbers in parentheses refer to Literature Cited, p. 71.

Foy (*5*), of New Zealand, reported that samples of oats are frequently found that do not give complete germination within the standard testing period of 12 days. He pointed out that almost invariably such samples are newly harvested. Foy's samples undoubtedly represent Red Algerian oats, a type that is grown almost exclusively in New Zealand.

Deming and Robertson (*2*) studied dormancy in three oat varieties. They observed (*2, p. 10*):

> There is considerable dormancy in Kanota oats (*Avena byzantina*) but little, if any, in the types of *Avena sativa* tested. The test agrees with field observations regarding sprouting in the shock. Both Colorado 37 and Nebraska 21 sprout readily in the shock under unfavorable weather conditions.

Johnson (*9, p. 283*) reported that in *Avena fatua*—

> (1) Great variations were found in the after-ripening periods of a number of *A. fatua* selections. (2) Evidence was obtained which strongly indicated that delayed germination is determined by a condition of the seed coat which develops after fertilization. (3) Results from tests of entire panicles indicated a correlation between germinability and the position of the seed in the panicle. (4) The after-ripening period of secondary grains was shown to be much longer than that of primary grains.

Hyde (*8, p. 363*) observed that in comparison with the fully ripened oats those incompletely after-ripened differed in three ways:

> Firstly, the optimum temperature for rapid germination was lower, being in the vicinity of 14° C. Secondly, the speed of germination at low temperatures was higher than that of fully after-ripened oats under the same conditions. Thirdly, at temperatures of 18° or 20° C. and higher, the germination of a proportion of the grain was greatly delayed.

Larson, Harvey, and Larson (*11*) studied the length of the dormant period in cereal seeds collected from the small-grain varietal plots at University Farm, St. Paul, Minn. They found that oats cut in the ripe stage generally had a short rest period. The Gopher variety completed its afterripening and germinated fully within 26 days. They tested early, midseason, and late varieties of common oats (*Avena sativa*) only. A rest period of any considerable length was found only in the late varieties.

MATERIALS AND METHODS

Twenty-five distinct varietal oat types were used in this experiment. These included both wild and cultivated varieties classed morphologically as belonging to six species and subspecies. Both winter and spring varieties of cultivated oats were included. The samples used were typical of the variety in each case, but only one sample of each was grown. It is recognized that there is some possibility that additional samples of these varieties grown under other conditions might have germinated differently than in the experiments reported here.

Most of the varieties tested were strictly spring types, the exceptions being Fulghum (H. C.[3] 726, C. I.[4] 3227), a strain of C. I. 708, Nortex, Fulghum (winter type, C. I. 2500), Aurora, Lee, Hairy Culberson, and Winter Turf. Of these Fulghum (C. I. 3227), Nortex, and Aurora may be grown from either fall or spring seeding, but the other four are grown only from fall seeding. Stanton (*14*) has re-

[3] H. C., an abbreviation of Hays Cereal, indicates the accession number of selections made at the Fort Hays Experiment Station, Hays, Kans.

[4] C. I. refers to accession number of the Division of Cereal Crops and Diseases (formerly Office of Cereal Investigations), U. S. Department of Agriculture.

cently reported on the origin and history of many of these varieties, including Brunker, Fulghum (C. I. 3227), Nortex, Fulghum (winter type, C. I. 2500), Coastblack, Richland, Black Mesdag, Victory, Miami, Cornellian, Markton, Aurora, Victoria, Lee, Hairy Culberson, and Liberty Hull-less. Murphy and Stanton (*12*) and Stanton and Murphy (*14A*) reported on the introduction of Berger and Victoria from South America. The strains of Early Champion, Victor, Winter Turf, White Tartar, and Storm King used were typical of the varietal types classified by Etheridge (*4*). Early Joanette is the only cultivated variety that has not been described in the literature. It is an early black oat of little economic importance, obtained from Illinois by the junior writer in following up an oat varietal survey made in 1919. Its origin is unknown, but on the basis of information available as to its characteristics it may have originated as a selection from Burt or some other variety of *Avena byzantina* C. Koch.

Ludoviciana (C. I. 1781) is a variety of the wild red oat that was obtained from the Mediterranean region about 25 years ago. It is typical of the type known as *Avena sterilis* var. *ludoviciana* (Durieu) Husnot, which does not have hairy awns such as *A. sterilis* var. *macrocarpa* (Moench) Briquet.

The common wild oat used in these investigations was received for varietal identification from a circus company of Allentown, Pa. The sample was taken from a carload of oats that had been screened from hard spring wheat and graded as feed oats. It is a typical gray-seeded variety of *Avena fatua*, and for convenience it has been referred to as the "Allentown" oat (C. I. 3322).

The oats tested were grown in the greenhouse at the Arlington Experiment Farm, Arlington, Va. Plants were harvested as soon as the upper leaves of the primary culms had begun to turn yellow, which occurred during the period from April 3 to May 1, 1933, in the different varieties. The seed was threshed at once, and a sample of straw of each variety was saved for moisture determination. The seed of each variety was divided into 50-kernel samples, placed in open paper containers, and kept in a screen-covered case in the laboratory. Toward the end of the test a few samples of less than 50 kernels were used, because of shortage of seed.

One sample of each variety was placed in the germinator immediately after harvest and the remaining samples at successive intervals of 3 to 4 days thereafter. The seed was germinated between blotters at a temperature of about 60° F. With a few exceptions germination counts were made at 3-day intervals. One sample of each variety was retained for moisture determination. It was weighed each time a new sample was placed in the germinator, and at the end of the test it was oven-dried and weighed.

In these tests any seed showing a protruding coleoptile was considered as having germinated. Ungerminated seed usually remained in the germinator until it rotted, or until the end of the test. The germinator was disinfected with a weak solution of formaldehyde to retard mold.

EXPERIMENTAL DATA

GERMINATION OF SPECIES AND VARIETIES

Germination tests were made on approximately 300 samples of seed of various ages of 25 varieties during the period from April 3 to June 22, 1933. The varieties are divided into the following four groups

according to the promptness of germination of the freshly harvested seed: (1) Prompt, (2) slow, (3) very slow, and (4) delayed, as shown in table 1. Freshly harvested seed or seed not over 4 days old of "prompt" varieties usually had started to germinate by the fifth day after harvest and within 5 to 7 days at least 80 percent had germinated. One variety classed as "prompt" (Liberty Hull-less) started to germinate the second day after harvest but did not germinate 100 percent until the twelfth day. Three-day-old seed of Early Champion germinated 100 percent in 7 days, although the germination of the freshly harvested seed was considerably delayed.

TABLE 1.—*Number of days for initial germination of 25 species and varieties of Avena stored for various periods after harvest*

Type of germination, species, and variety	C. I. No.	Time required for germination after storage for—												
		0 days	2–4 days	4–7 days	8–10 days	11–13 days	14–16 days	17–19 days	19–21 days	22–25 days	26–28 days	36–39 days	47–50 days	74–80 days
Prompt:														
A. sativa:														
Black Mesdag	1877	4	5	3	4	3	3	3	4	2	3	2	4	-----
Cornellian	1842	3	3	4	2	3	3	3	3	3	2	3	3	-----
Early Champion	1623	6	3	7	4	2	3	3	3	3	3	2	3	-----
Hairy Culberson (winter oat)	2505	3	5	3	3	3	3	2	2	3	5	3	3	-----
Miami	2245	3	3	3	4	2	3	3	3	3	3	2	3	-----
Victory	1145	6	7	4	2	3	3	3	3	3	4	3	3	-----
Winter Turf (winter oat)	1570	5	2	6	6	3	3	4	5	4	5	-----	-----	-----
A. nuda:														
Liberty Hull-less	845	2	3	4	3	3	3	4	2	3	3	2	3	-----
A. byzantina:														
Fulghum (winter oat)	2500	5	2	3	6	3	5	4	5	4	4	3	3	-----
Slow:														
A. sativa:														
Aurora	831	6	6	6	7	4	5	3	6	3	3	4	3	-----
Richland	787	6	5	6	4	6	6	3	4	2	3	2	2	-----
A. sativa var. *orientalis* (Schreb.) Alefeld:														
Storm King	1602	9	6	6	7	4	5	6	6	3	3	4	-----	-----
A. fatua:														
"Allentown"	3322	4	5	6	7	6	6	7	4	5	6	5	4	-----
Very slow:														
A. sativa:														
Markton	2053	8	27	56	-----	6	3	4	2	3	3	2	3	-----
Lee (winter oat)	2042	15	12	8	6	6	6	3	3	4	5	5	-----	-----
Victor	803	12	9	6	7	4	5	3	3	6	3	2	3	-----
A. sativa var. *orientalis:*														
White Tartar	1614	27	13	6	7	4	2	3	3	3	3	2	3	-----
A. byzantina:														
Brunker	2054	18	6	5	6	4	6	6	7	4	5	4	2	-----
Fulghum (H. C. 726)	3227	15	11	12	10	6	6	7	9	8	6	5	4	-----
A. sterilis L.:														
A. sterilis var. *ludoviciana*	1781	14	12	7	6	6	7	4	8	6	3	4	5	-----
Delayed:														
A. sativa:														
Early Joanette	1092	64	56	14	12	66	62	52	59	49	14	4	9	3
Victoria [1]	2401	68	66	27	6	21	6	8	6	6	6	2	3	-----
A. byzantina:														
Berger	2926	29	43	53	8	6	6	3	6	5	4	5	10	-----
Coastblack	1025	56	12	8	9	6	6	3	5	7	5	5	7	-----
Nortex	2382	(2)	(2)	62	10	56	6	5	6	3	6	7	5	3

[1] A more or less intermediate type that may be classified as belonging to either *Avena sativa* or *A. byzantina*, depending on characters considered.
[2] Did not germinate throughout duration of test, ended June 22.

The percentage germination for those varieties classified as germinating promptly is given in table 2.

TABLE 2.—*Percentage germination of samples of 9 promptly germinating varieties of Avena stored for various periods after harvest*

Variety	Period of storage	Germination after being in germinator for—													
		4 days	7 days	10 days	13 days	16 days	19 days	22 days	25 days	28 days	31 days	41 days	51 days	61 days	71 days
	Days	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Black Mesdag (*A. sativa*, early)	0	56	94	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	4	0	62	72	74	76	88	-----	-----	90	-----	-----	-----	98	-----
	6	2	88	-----	90	94	-----	-----	-----	-----	-----	-----	-----	100	-----
	8	24	94	96	-----	-----	-----	-----	-----	-----	98	-----	-----	-----	-----
	12	2	90	92	-----	-----	94	-----	-----	96	-----	98	-----	-----	-----
	15	2	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	18	8	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	21	96	98	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	25	84	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	28	10	98	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	38	40	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	48	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Early Champion (*A. sativa*, early)	0	0	44	58	60	-----	62	-----	-----	-----	-----	66	96	-----	100
	3	10	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	6	0	82	84	-----	86	92	-----	-----	94	-----	-----	96	100	-----
	9	70	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	13	14	98	-----	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	15	18	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	18	66	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	21	56	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	24	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	27	65	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	39	50	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	49	90	-----	-----	95	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Cornellian (*A. sativa*, midseason)	0	44	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	3	38	94	-----	-----	-----	-----	-----	-----	-----	-----	96	-----	100	-----
	6	88	98	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	10	42	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	12	30	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	15	90	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	18	40	98	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	21	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	24	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	27	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	38	90	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	49	76	-----	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Miami (*A. sativa*, midseason)	0	40	94	96	98	-----	-----	-----	100	-----	-----	-----	-----	-----	-----
	3	8	96	98	-----	100	-----	-----	-----	-----	-----	-----	-----	-----	-----
	6	68	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	9	94	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	13	40	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	15	38	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	18	46	98	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	21	16	98	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	24	90	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	27	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	39	70	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	49	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Victory (*A. sativa*, midseason)	0	0	90	-----	-----	-----	92	94	96	-----	100	-----	-----	-----	-----
	3	0	16	-----	18	24	30	40	52	54	68	98	100	-----	-----
	6	56	80	-----	88	90	-----	94	96	100	-----	-----	-----	-----	-----
	10	26	92	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	12	48	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	15	76	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	18	52	98	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	21	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	24	90	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	27	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	38	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	49	68	-----	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Hairy Culberson (*A. sativa*, winter)	0	20	54	74	84	90	92	-----	94	-----	-----	96	98	-----	-----
	3	0	72	82	88	-----	-----	-----	-----	-----	-----	-----	98	-----	-----
	5	28	82	98	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	8	66	96	-----	-----	-----	-----	-----	-----	-----	-----	98	-----	-----	-----
	11	76	92	98	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	14	60	94	-----	-----	-----	-----	-----	-----	-----	-----	-----	98	-----	-----
	17	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	19	50	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	22	90	95	100	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	26	75	95	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	36	64	72	-----	78	84	-----	-----	-----	-----	-----	-----	-----	-----	-----
	46	80	98	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

TABLE 2.—*Percentage germination of samples of 9 promptly germinating varieties of Avena stored for various periods after harvest*—Continued

Variety	Period of storage	Germination after being in germinator for—													
		4 days	7 days	10 days	13 days	16 days	19 days	22 days	25 days	28 days	31 days	41 days	51 days	61 days	71 days
	Days	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Winter Turf (*A. sativa*, winter)	0	0	4	62	90	100									
	3	2	38	82	96	98						100			
	5	0	62	96		100									
	8	0	88	92	94				96	98					
	11	2	34	78	82			98							
	14	2	90			94	96								
	17	60	100												
	19	0	75		85	95									
	22	10		90	100										
	26	0	100												
Fulghum, C. I. 2500 (*A. byzantina*, winter)	0	0	2	66	90	100									
	3	2	26	92	96								100		
	5	2	34	96			98			100					
	8	0	100												
	11	4	60	80	86			100							
	14	0	90		100										
	17	25	85												
	19	0	85	90	95	100									
	22	10	60												
	26	0	100												
	36	4	32		58	88									
	46	22	100												
Liberty Hull-less (*A. nuda*, midseason)	0	4	30	48	56	88	94	96		98	100				
	2	88	94	96	100										
	4	88	92	96	98				100						
	8	54	76	86	100										
	11	100													
	14	100													
	17	100													
	21	100													
	23	100													
	26	100													
	38	100													
	49	100													

Freshly harvested seed of the *Avena sativa* varieties, Black Mesdag, Cornellian, Miami, Victory, Hairy Culberson, Winter Turf, and Early Champion, germinated promptly, as did also one variety of *A. byzantina*, Fulghum (winter type), and the one variety of *A. nuda* L., Liberty Hull-less.

The germination of varieties classed as "slow" is shown in table 3. These usually had started to germinate by the sixth day after harvest, and seed 2 to 4 days old started germinating promptly. Varieties classed as slow included Richland, Early Champion, and Aurora, belonging to *Avena sativa*, the *A. sativa* var. *orientalis* variety Storm King, and the only *A. fatua* strain tested. No variety classified morphologically as belonging to *A. byzantina* was included in this germination group.

Seed of varieties classed as "very slow" (table 4) started to germinate in from 8 to 27 days; seed 7 days old germinated promptly. These varieties included Markton, Lee, and Victor, classed morphologically as belonging to *Avena sativa;* White Tartar, belonging to *A. sativa* var. *orientalis;* Brunker and Fulghum (C. I. 3227), belonging to *A. byzantina*.

The varieties classed as "delayed" (table 5) did not germinate effectively until about 2 months after harvest, regardless of whether the seed was in the germinator or in dry storage, although partial germination occurred earlier in some samples. The varieties in this group are Early Joanette and Victoria, classified morphologically as belonging to *Avena sativa*, and Berger, Nortex, and Coastblack, belonging to *A. byzantina*, and the strain of *A. sterilis* var. *ludoviciana*.

These results offer an explanation of why poor field stands sometimes are obtained from seed of strains of Red Rustproof oats, such as Appler and Nortex, when sown in the South in August for early fall pasturage.

TABLE 3.—*Percentage germination of samples of 4 slow-germinating varieties of Avena stored for various periods after harvest*

Variety	Period of storage	Germination after being in germinator for—														
		4 days	7 days	10 days	13 days	16 days	19 days	22 days	25 days	28 days	31 days	41 days	51 days	61 days	71 days	81 days
	Days	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Aurora (*A. sativa*, early)	0	0	4	6	----	----	----	----	----	10	16	24	26	28	44	----
	3	0	16	18	20	----	----	24	32	----	34	42	48	----	62	----
	6	0	48	----	----	----	----	----	50	----	52	----	56	58	----	----
	9	0	26	28	30	34	----	36	38	----	----	----	----	46	----	----
	12	6	50	54	56	----	60	62	----	64	----	----	----	68	----	----
	16	0	44	68	----	76	----	----	----	----	----	80	90	----	----	----
	18	10	70	----	----	----	----	----	----	----	----	----	----	----	----	----
	21	0	100	----	----	----	----	----	----	----	----	----	----	----	----	----
	24	4	96	----	----	----	----	----	----	----	----	98	----	----	----	----
	27	96	100	----	----	----	----	----	----	----	----	----	----	----	----	----
	38	100	----	----	----	----	----	----	----	----	----	----	----	----	----	----
	49	100	----	----	----	----	----	----	----	----	----	----	----	----	----	----
Richland (*A. sativa*, early)	0	0	2	4	6	8	12	----	----	----	16	38	46	64	72	100
	4	0	2	6	10	20	28	30	32	----	36	48	50	68	100	----
	6	0	50	54	60	64	68	72	----	----	76	----	84	88	98	----
	8	14	46	54	56	----	62	----	----	68	----	74	----	----	94	----
	12	0	38	42	----	46	----	50	----	----	----	54	62	----	78	----
	15	0	100	----	----	----	----	----	----	----	----	----	----	----	----	----
	18	28	92	94	----	96	----	----	----	----	----	----	----	98	----	----
	21	82	88	92	98	100	----	----	----	----	----	----	----	----	----	----
	25	16	80	96	98	100	----	----	----	----	----	----	----	----	----	----
	27	70	100	----	----	----	----	----	----	----	----	----	----	----	----	----
	37	100	----	----	----	----	----	----	----	----	----	----	----	----	----	----
	47	10	100	----	----	----	----	----	----	----	----	----	----	----	----	----
Storm King (*A. sativa* var. *orientalis*, late)	0	0	----	4	----	20	----	----	----	----	----	----	----	24	56	----
	3	0	40	60	----	----	64	----	----	68	----	----	80	84	88	----
	6	0	80	88	92	----	----	96	----	----	----	----	----	100	----	----
	9	0	84	88	92	96	----	----	----	----	----	----	----	----	----	----
	12	8	72	----	----	80	----	84	88	----	----	96	----	----	----	----
	16	0	68	88	----	----	----	----	----	----	----	----	----	----	----	----
	18	0	80	90	----	----	100	----	----	----	----	----	----	----	----	----
	21	0	70	----	----	80	----	----	----	----	----	----	----	----	----	----
	24	10	100	----	----	----	----	----	----	----	----	----	----	----	----	----
	27	100	----	----	----	----	----	----	----	----	----	----	----	----	----	----
	38	80	----	----	----	----	----	----	----	----	----	----	----	----	----	----
A. fatua ("Allentown," early)	0	2	36	58	66	72	74	84	----	----	----	----	----	----	----	----
	4	0	50	88	94	----	96	----	98	----	----	----	----	----	----	----
	6	0	54	82	84	90	----	----	----	----	----	----	----	----	----	----
	8	0	48	----	66	----	68	70	74	----	----	----	----	----	----	----
	12	0	80	88	----	----	----	90	----	----	----	----	----	----	----	----
	15	0	74	76	----	----	----	80	----	----	----	----	----	----	----	----
	18	0	70	72	74	76	----	----	----	----	----	----	----	----	----	----
	21	46	66	74	76	84	----	----	----	----	----	----	----	----	----	----
	25	0	48	70	76	----	----	78	----	----	----	----	----	----	----	----
	27	0	100	----	----	----	----	----	----	----	----	----	----	----	----	----
	37	0	80	84	92	----	----	----	96	----	----	----	----	----	----	----
	47	100	----	----	----	----	----	----	----	----	----	----	----	----	----	----

TABLE 4.—*Percentage germination of samples of 6 very slow-germinating varieties of Avena stored for various periods after harvest*

Variety	Period of storage	Germination after being in germinator for—													
		4 days	7 days	10 days	13 days	16 days	19 days	22 days	25 days	28 days	31 days	41 days	51 days	61 days	71 days	81 days
	Days	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Markton (*A. sativa*, midseason)	0	0	----	4	----	----	----	----	----	----	----	----	----	----	42	52
	2	0	----	----	----	----	----	----	----	2	----	----	----	4	46	----
	4	0	----	----	----	----	----	----	----	----	----	----	----	2	10	----
	8	0	4	----	----	----	----	6	----	----	8	----	----	----	20	----
	11	0	60	----	----	----	62	----	----	----	----	----	----	----	----	----
	14	6	56	62	66	----	----	----	----	----	68	82	----	90	----	----
	17	8	22	24	26	28	30	----	32	----	----	38	----	----	64	----
	21	8	68	92	94	----	----	----	----	----	----	96	98	----	----	----
	23	20	80	100	----	----	----	----	----	----	----	----	----	----	----	----
	26	4	94	96	----	----	----	----	----	----	----	----	100	----	----	----
	38	100	----	----	----	----	----	----	----	----	----	----	----	----	----	----
	49	90	95	----	100	----	----	----	----	----	----	----	----	----	----	----
Victor (*A. sativa*, late)	0	0	----	----	4	8	----	----	10	12	----	----	14	26	80	----
	3	0	----	16	20	28	30	34	36	40	----	----	52	90	----	----
	6	0	64	72	74	76	80	82	----	----	86	----	----	92	----	----
	9	0	66	74	82	88	----	90	----	----	----	92	----	100	----	----
	12	6	54	66	74	----	78	80	----	84	----	86	92	----	----	----
	16	0	66	94	----	96	----	----	----	----	----	98	----	----	----	----
	18	2	92	98	----	----	----	----	----	----	----	100	----	----	----	----
	21	6	90	100	----	----	----	----	----	----	----	----	----	----	----	----
	24	0	98	100	----	----	----	----	----	----	----	----	----	----	----	----
	27	90	100	----	----	----	----	----	----	----	----	----	----	----	----	----
	38	100	----	----	----	----	----	----	----	----	----	----	----	----	----	----
	49	100	----	----	----	----	----	----	----	----	----	----	----	----	----	----
Lee (*A. sativa*, winter oat)	0	0	----	----	----	2	6	20	----	24	26	30	32	86	----	----
	3	0	----	----	16	34	40	44	----	46	----	52	----	92	----	----
	7	0	----	32	56	68	70	72	74	76	----	80	100	----	----	----
	9	0	26	50	66	70	82	84	----	----	86	92	----	----	----	----
	12	0	76	92	----	----	----	----	----	----	----	----	98	----	----	----
	15	0	84	88	----	----	----	----	92	94	----	98	----	----	----	----
	18	26	46	56	----	----	----	58	66	68	----	100	----	----	----	----
	21	2	88	----	90	----	94	96	----	98	----	----	----	----	----	----
	24	60	96	----	100	----	----	----	----	----	----	----	----	----	----	----
	26	0	85	----	95	----	100	----	----	----	----	----	----	----	----	----
	38	0	100	----	----	----	----	----	----	----	----	----	----	----	----	----
	49	0	100	----	----	----	----	----	----	----	----	----	----	----	----	----
White Tartar (*A. sativa* var. *orientalis*, late)	0	0	----	----	----	----	----	----	----	4	12	32	38	52	72	----
	3	0	----	----	2	----	6	24	40	52	56	58	72	80	84	----
	6	0	72	80	86	----	88	90	----	92	----	----	----	100	----	----
	9	0	78	88	----	92	----	96	----	----	98	----	----	----	----	----
	12	32	50	56	62	64	70	----	72	----	----	76	----	82	----	----
	16	20	80	90	92	94	----	----	----	----	----	----	----	----	----	----
	18	16	84	96	98	----	----	----	----	----	----	----	----	----	----	----
	21	22	88	90	94	----	----	----	----	----	----	----	----	----	----	----
	24	36	94	----	----	----	----	----	----	----	----	----	----	----	----	----
	27	90	95	----	100	----	----	----	----	----	----	----	----	----	----	----
	38	70	----	100	----	----	----	----	----	----	----	----	----	----	----	----
	49	100	----	----	----	----	----	----	----	----	----	----	----	----	----	----
Brunker (*A. byzantina*, early)	0	0	----	----	----	----	6	12	48	----	54	72	84	96	100	----
	3	0	8	----	----	12	20	28	34	36	44	74	90	100	----	----
	7	0	4	18	20	22	32	----	----	34	38	62	78	100	----	----
	9	0	26	----	30	34	----	----	----	36	46	66	78	100	----	----
	11	2	36	44	50	----	----	54	56	----	66	80	84	100	----	----
	15	0	22	28	----	----	30	----	34	42	----	46	100	----	----	----
	18	0	76	90	----	----	----	92	96	----	98	----	100	----	----	----
	21	0	60	68	70	78	86	----	----	----	----	----	100	----	----	----
	24	22	90	92	----	----	94	----	----	----	----	96	98	----	----	----
	29	0	66	92	98	100	----	----	----	----	----	----	----	----	----	----
	39	100	----	----	----	----	----	----	----	----	----	----	----	----	----	----
	51	100	----	----	----	----	----	----	----	----	----	----	----	----	----	----
Fulghum, C. I. 3227 (*A. byzantina*, early)	0	0	----	----	----	2	4	8	----	----	----	----	12	38	----	80
	4	0	----	----	----	4	6	----	----	----	----	16	20	34	76	----
	6	0	----	----	2	----	----	----	----	----	10	12	20	34	40	----
	8	0	----	2	----	----	----	----	----	6	----	10	16	26	62	----
	12	0	2	4	----	----	----	----	----	----	6	10	12	14	64	----
	15	0	26	----	34	----	----	----	----	----	----	36	38	80	----	----
	18	0	6	----	----	8	12	----	----	16	----	18	34	62	----	----
	21	0	----	4	6	----	----	----	----	8	----	16	66	80	----	----
	25	0	----	22	----	24	----	26	----	28	----	46	84	----	----	----
	27	0	40	60	80	----	----	----	----	----	----	----	100	----	----	----
	37	0	48	64	----	----	66	70	72	----	----	80	----	----	----	----
	47	10	20	----	----	30	----	----	----	----	----	----	----	----	----	----

TABLE 5.—*Percentage germination of samples of 6 varieties of Avena showing delayed germination, stored for various periods after harvest*

Variety	Period of storage	Germination after being in germinator for—														
		4 days	7 days	10 days	13 days	16 days	19 days	22 days	25 days	28 days	31 days	41 days	51 days	61 days	71 days	81 days
	Days	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Early Joanette (*A. sativa*, early)	0	0	----	----	----	----	----	----	----	----	----	----	----	----	30	80
	3	0	----	----	----	----	----	----	----	----	----	----	----	14	----	46
	7	0	----	----	----	4	----	----	----	6	----	8	----	26	56	----
	9	0	----	----	2	8	----	10	----	12	----	----	----	----	86	----
	11	0	----	----	----	----	----	----	----	----	----	----	----	----	16	----
	15	0	----	----	----	----	----	----	----	----	----	----	----	----	26	----
	18	0	----	----	----	----	----	----	----	----	----	----	2	26	32	----
	21	0	----	----	----	----	----	----	----	----	----	----	----	2	----	----
	24	0	----	----	----	----	----	----	----	----	----	----	4	20	----	----
	28	0	----	----	----	5	45	----	----	----	----	----	80	85	----	----
	38	5	----	----	----	10	----	15	----	----	----	----	----	----	----	----
	50	0	----	25	45	75	80	----	----	----	----	----	----	----	----	----
	74	70	100	----	----	----	----	----	----	----	----	----	----	----	----	----
Victoria (*A. sativa*, late)	0	0	----	----	----	----	----	----	----	----	----	----	----	----	40	----
	2	0	----	----	----	----	----	----	----	----	----	----	----	----	52	----
	6	0	----	----	----	----	----	----	----	2	----	----	6	----	48	----
	9	0	28	32	----	----	----	34	----	----	----	----	----	40	----	----
	12	0	----	----	----	----	----	2	----	----	----	----	4	24	----	----
	15	0	2	4	----	20	24	30	34	----	----	38	44	84	----	----
	19	0	----	10	28	34	54	60	62	----	66	74	82	----	----	----
	21	0	20	40	----	50	----	----	----	----	60	70	----	----	----	----
	24	0	60	65	----	----	----	----	----	----	----	----	----	----	----	----
	27	0	35	----	75	----	----	----	----	80	----	----	----	----	----	----
	36	75	85	----	----	90	----	----	----	----	----	----	----	----	----	----
	47	25	75	80	----	----	----	95	----	----	----	----	----	----	----	----
Berger (*A. byzantina*, midseason)	0	0	----	----	----	----	----	----	4	----	8	----	----	60	----	----
	3	0	----	----	----	----	----	----	----	----	----	----	5	20	----	----
	6	0	----	----	----	----	----	----	----	----	----	----	----	25	----	----
	10	0	----	15	30	45	----	50	----	----	----	70	75	----	----	----
	12	0	20	30	50	----	60	----	----	90	----	----	----	----	----	----
	15	0	40	50	----	----	----	----	----	----	----	----	80	----	----	----
	18	10	80	----	90	----	----	----	----	----	----	100	----	----	----	----
	21	0	50	85	----	----	----	----	----	----	----	95	----	----	----	----
	24	0	75	----	----	----	80	95	----	----	100	----	----	----	----	----
	27	10	90	----	----	100	----	----	----	----	----	----	----	----	----	----
	38	0	80	90	----	----	----	95	----	----	----	----	----	----	----	----
	49	0	20	100	----	----	----	----	----	----	----	----	----	----	----	----
Nortex (*A. byzantina*, midseason)	0	0	----	----	----	----	----	----	----	----	----	----	----	----	----	0
	4	0	----	----	----	----	----	----	----	----	----	----	----	----	----	0
	8	0	----	----	----	----	----	----	----	----	----	----	----	----	8	----
	11	0	----	4	----	----	----	----	----	----	----	----	----	8	----	----
	14	0	----	----	----	----	----	----	----	----	----	----	----	4	----	----
	17	0	4	----	----	6	----	----	----	----	----	----	----	38	----	----
	21	0	2	12	14	16	18	----	----	----	----	20	58	60	----	----
	23	0	----	----	10	----	----	----	----	----	----	40	50	----	----	----
	26	15	20	----	----	----	----	----	----	----	----	----	45	----	----	----
	29	0	35	----	----	40	----	----	----	----	----	60	70	----	----	----
	37	0	35	----	----	45	55	----	----	----	----	----	----	----	----	----
	48	0	20	25	30	----	----	----	----	----	----	----	----	----	----	----
	66	20	100	----	----	----	----	----	----	----	----	----	----	----	----	----
Coastblack (*A. byzantina*, late)	0	0	----	----	----	----	----	----	----	----	----	----	----	24	----	----
	3	0	----	----	2	----	4	----	----	----	----	20	24	82	----	----
	7	0	----	4	28	56	58	----	----	----	60	68	90	----	----	----
	9	0	----	70	90	----	----	----	----	----	----	100	----	----	----	----
	12	0	42	66	----	68	----	----	----	----	----	----	76	----	----	----
	15	0	84	86	88	90	92	----	----	----	----	96	100	----	----	----
	18	10	42	64	70	----	----	----	78	80	----	84	----	----	----	----
	21	0	66	----	80	----	86	90	96	----	98	100	----	----	----	----
	24	0	10	25	----	90	----	----	----	----	----	----	----	----	----	----
	26	0	25	35	40	75	100	----	----	----	----	----	----	----	----	----
	38	0	100	----	----	----	----	----	----	----	----	----	----	----	----	----
	49	0	92	----	----	----	----	----	----	----	----	----	----	----	----	----
A. sterilis var. *ludoviciana* (midseason)	0	0	----	----	----	12	20	24	----	----	----	26	28	42	54	64
	2	0	----	----	2	6	10	----	----	12	16	22	26	28	42	44
	4	0	2	----	----	----	4	----	----	10	12	18	22	34	60	----
	8	0	2	6	----	10	16	20	28	30	----	32	34	----	60	----
	11	0	56	62	64	72	----	78	----	----	80	82	84	98	----	----
	14	0	2	6	10	14	----	16	18	20	----	22	30	36	----	----
	17	4	----	12	20	26	30	34	36	40	----	----	----	----	----	----
	21	0	----	32	50	60	74	76	----	----	78	82	84	----	----	----
	23	0	10	40	60	----	70	----	----	----	80	----	----	----	----	----
	26	10	70	----	----	----	80	----	----	----	----	----	----	----	----	----
	38	55	80	----	----	85	----	----	----	----	----	----	----	----	----	----
	49	0	40	55	60	65	----	70	----	----	----	----	----	----	----	----

The data in general show that the varieties classed morphologically as belonging to *Avena sativa* varied widely, exhibiting all degrees of slowness or rapidity in germination. One variety classed as belonging to *A. byzantina* was prompt in germination, but the other varieties of this species showed more or less dormancy. Varieties of *A. sativa orientalis* were also inclined to be slow to germinate. Among the wild species, *A. sterilis*, the supposed progenitor of *A. byzantina*, was very slow in germinating, and *A. fatua*, the supposed progenitor of *A. sativa*, was classed as "slow."

There is a possibility that some of the varieties classed morphologically as belonging to *Avena sativa* that exhibited more or less dormancy in these tests may have originated from hybrids with *A. byzantina*. Markton, Victoria, and Early Joanette have characteristics other than slow germination that indicate that they may have been derived in part from this species.

Other varieties classed as belonging to *Avena sativa* that give some indications of dormancy are Victor, Richland, Aurora, and Lee. Aurora was originated as a selection from Red Rustproof, and Lee as a hybrid between Aurora and Winter Turf, hence their *A. byzantina* derivation is established. This suggests that dormancy may be helpful as an indicator of the probable origin and relationship of oat varieties.

RELATION OF KERNEL CHARACTERS TO GERMINATION

Several investigators have indicated that certain morphological characters of the oat kernel are associated with dormancy. Table 6 contains a tabular description of the principal distinguishing morphological characters of the oat kernel of the varieties and strains included in these experiments.

Oats having the 5 recognizable kernel colors were tested in the following numbers: Black, 4; gray, 4; red, 6; yellow, 4; and white, 6. Variation in promptness to germinate occurred in all color classes of cultivated oats, but except for Victoria, an oat of reddish-gray color, only black and red oat varieties were classed as having "delayed" germination. Of 10 black or red varieties of cultivated oats, 8 were very slow or delayed and 2 were prompt in germination. *Avena fatua* (gray) and *A. sterilis* (red) were slow in germination. Varieties having the darker-colored kernels appear more frequently to have delayed germination.

Oat varieties may have one or more awns per spikelet in almost all panicles or awns few to almost entirely lacking. The awns may be straight or twisted. Varieties in each germination class included some classed as awnless and some having twisted awns and straight awns. It would seem that awn type is not an index of dormancy in oats.

The type of basal scars in oat kernels may be classed as prominent, obscure, or absent. Of the five strains classed as having prominent basal scars, germination was slow or delayed in all.

Oat kernels with prominent basal scars shatter readily, and varieties of this type probably would not be perpetuated in nature without some degree of dormancy.

The mode of separation between the primary and secondary kernels of the oat spikelet has been described as abscission in *Avena sativa* varieties, fracture in *A. sterilis*, and intermediate in certain varieties

having characteristics of both species (*1A*). Seventeen of the varieties included in these experiments have the *sativa* type of separation or articulation, four varieties were classed as separating by fracture, as in *A. sterilis*, and three as intermediate. The data indicate that dormancy is most often observed in oats having a fracture or intermediate separation. Only one variety so classed germinated promptly. Varieties separating by abscission may or may not have delayed germination.

TABLE 6.—*Kernel characters and type of germination of 25 varieties of Avena*

Species and variety	Kernel characters					Germination
	Type of basal scar	Articulation	Color	Awning	Basal hairs	
Wild species:						
A. fatua, "Allentown."	Prominent	Sativa	Gray	Twisted, geniculate.	Many	Slow.
A. sterilis var. *ludoviciana*	do	Sterilis	Red	do	do	Delayed.
Cultivated species:						
A. byzantina:						
Berger	do	do	do	Straight	do	Do.
Brunker	Obscure	Intermediate	do	Straight and absent.	Few	Very slow.
Coastblack	Prominent	Sterilis	Black	Straight	Many	Delayed.
Fulghum selection (C. I. 3227).	Obscure	Intermediate	Red	Straight and absent.	Few	Very slow.
Fulghum (winter type).	do	do	do	do	do	Prompt.
Nortex	Prominent	Sterilis	do	Straight	Many	Delayed.
A. sativa:						
Aurora	Absent	Sativa	Yellow	Absent	Few	Slow.
Black Mesdag	Obscure	do	Black	Twisted, geniculate.	do	Prompt.
Cornellian	Absent	do	Gray	Absent	do	Do.
Early Champion	Obscure	do	White	Twisted, geniculate.	do	Do.
Early Joanette	do	do	Black	do	Many	Delayed.
Hairy Culberson (winter oat).	Absent	do	Gray	do	Few	Prompt.
Lee (winter oat)	do	do	Yellow	Absent	do	Very slow.
Markton	Obscure	do	do	Twisted, geniculate.	do	Do.
Miami	do	do	White	do	do	Prompt.
Richland	do	do	Yellow	Absent to twisted, subgeniculate.	do	Slow.
Victor	do	do	Black	Twisted, geniculate.	Absent	Very slow.
Victoria	do	do	Gray	do	Few	Delayed.
Victory	Absent	do	White	Straight and twisted, geniculate.	Absent	Prompt.
Winter Turf (winter oat).	do	do	Gray	Twisted, geniculate.	Few	Do.
A. sativa var. *orientalis:*						
Storm King	do	do	White	Absent	Absent	Slow.
White Tartar	do	do	do	do	do	Do.
A. nuda:						
Liberty Hull-less	do		do	Twisted, geniculate.		Prompt.

Of the 25 varieties tested, 4 were classed as having no basal hairs, and *Avena nuda* could not be studied for this character. Four of the five oat varieties classed as having delayed germination had many basal hairs, and the fifth had a few. It has been shown, however, that at least in some oat types, notably fatuoids, having a prominent basal cavity and numerous hairs, delayed germination may not occur.

Dark-colored kernels, prominent basal scars, and numerous basal hairs, that often accompany slow or delayed germination, are frequent-

ly found in the species *Avena fatua*, *A. sterilis*, and *A. byzantina*, in which slow or delayed germination usually occurs. However, apparent relationship of these characters to dormancy may be merely incidental.

RELATION OF KERNEL SIZE TO GERMINATION

Table 7 presents data on size of kernel and percentage of groat in kernels of the oat varieties included in this experiment. The varieties are listed in the order of the weight per 100 kernels.

TABLE 7.—*Kernel weight, percentage of groat, kernel length, and type of germination of 25 varieties of Avena*

Variety	Oven-dry weight of 100 kernels	Groat	Length of kernel	Type of germination
	Grams	*Percent*	*Mm*	
A. fatua, "Allentown"	1.53	63.0		Slow.
A. sterilis var. *ludoviciana*	1.62	58.5		Delayed.
Early Joanette	2.16	82.5	16	Do.
White Tartar	2.19	76.4		Very slow.
Richland	2.26	78.0	17	Slow.
Victoria	2.31	71.1	20	Delayed.
Early Champion	2.40	82.2		Prompt.
Lee	2.44	80.7	16	Very slow.
Black Mesdag	2.59	75.0	19	Prompt.
Cornellian	2.59	80.4	18	Do.
Hairy Culberson	2.62	80.6	15	Do.
Aurora	2.74	83.7		Slow.
Liberty Hull-less	[1] 2.78			Prompt.
Fulghum (C. I. 3227)	2.99	80.2		Very slow.
Victory	3.04	78.2		Prompt.
Winter Turf	3.09	86.3	17	Do.
Fulghum (winter type)	3.12	82.6	17	Do.
Brunker	3.31	75.8	17	Very slow.
Berger	3.48	76.8	18	Delayed.
Victor	3.51	72.2	19	Very slow.
Miami	3.54	78.3		Prompt.
Markton	3.59	77.6	18	Very slow.
Nortex	3.80	80.9	18	Delayed.
Coastblack	4.08	77.7		Do.
Storm King	4.26	70.4		Slow.

[1] Groat only.

On the basis of weight, small- and large-kerneled varieties more frequently exhibited dormancy than varieties with kernels of medium size. The nine varieties that germinated promptly had midsized kernels weighing from 2.40 to 3.54 g per 100 kernels. Liberty Hull-less was included in this group, although, on the basis of groat weight, its kernels might be classed as large.

The highest percentage of groat occurs in the varieties having midsized kernels, and in this group, as just shown, a large percentage of the varieties are prompt in germination. This might indicate that heavy hulls are related to dormancy were it not for certain exceptions among the varieties having delayed germination, such as Nortex, Early Joanette, and Coastblack.

Apparently no relation exists between delayed germination and kernel length, kernel weight, or percentage of groat.

RELATION OF GROWTH HABIT, COLD RESISTANCE, AND MATURITY TO GERMINATION

Among the five varieties of winter oats, germination was prompt in Hairy Culberson, Winter Turf, and Fulghum (winter type, C. I. 2500), very slow in Lee, and delayed in Coastblack.

Additional varieties included in these tests that are extensively grown from fall seeding are Nortex and Fulghum (C. I. 708), of which C. I. 3227 is a selection. Germination was very slow in the strain of Fulghum and delayed in Nortex. Hairy Culberson and a Fulghum (winter type) strain were recently reported by Coffman (*1*) to be more hardy than Winter Turf. These data indicate that delayed germination in freshly harvested oats is not associated with winter growth habit or cold resistance as found in the hardier winter types.

Spring oat varieties ranging in maturity from the earliest to the latest were tested. Among early-maturing cultivated varieties germination was prompt in Black Mesdag and Early Champion and slow in Aurora and Richland, very slow in Brunker and Fulghum (C. I. 3227), and delayed in Early Joanette. Midseason varieties were similarly widely distributed among different germination classes. Cornellian, Miami, and Victory germinated promptly; Markton was very slow; and germination was delayed in Berger and Nortex, the latter a variety grown both from spring and fall seeding. Several late-maturing varieties were tested. Germination was slow in Storm King, very slow in Victor and White Tartar, and delayed in Victoria.

Liberty Hull-less, a midseason oat, germinated promptly. The early-maturing strain of *Avena fatua* tested germinated slowly, and the midseason strain of *A. sterilis* germinated very slowly. The data presented indicate that delayed germination is not necessarily related to time of maturity in oats.

GERMINATION OF PRIMARY AND SECONDARY KERNELS

In recording the data on germination it soon became evident that the primary kernels of the spikelet frequently germinated before the secondary kernels. Thereafter the germination of primary and secondary kernels was recorded separately, and these data are presented in table 8. The weighted average germination of freshly harvested seed of the four prompt-germinating varieties, Cornellian, Miami, Victory, and Early Champion, that eventually germinated 100 percent was 33 percent for the primary kernels, 20 percent for the secondary kernels within 4 days, and 97 and 91 percent, respectively, within 7 days. There was some indication that the secondary kernels germinate relatively more promptly after storage for 6 days or more following harvest than they do within 3 days after harvest.

RELATION OF MOISTURE CONTENT TO GERMINATION

A relationship has been reported between moisture content and dormancy in seeds. Data on the percentage of moisture in the seed and straw of freshly harvested oats and in the seed 3 or 4 days later are presented in table 9.

The data show wide variations in the moisture content of oat varieties in each germination class at the time the seed was harvested. Twelve of the twenty-five varieties had less than 15 percent moisture in the seed at harvest. Varieties having the highest moisture content were not necessarily those slowest to germinate among either the wild or the cultivated species. Seed of freshly harvested Black Mesdag, which germinated promptly, contained 16.3 percent moisture, whereas seed of Coastblack containing 15.3 percent, Berger 13.3 percent, Lee 12.1 percent, and Markton 13.8 percent were delayed or very slow in germination.

TABLE 8.—*Germination of primary and secondary kernels of 12 varieties of Avena*

Variety	Age of seed	Germination							
		3 or 4 days		6 or 7 days		9 to 15 days		24 to 69 days	
		Primary	Secondary	Primary	Secondary	Primary	Secondary	Primary	Secondary
	Days	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Cornellian	0	44	44	100	100				
	3	48	15	100	79			100	100
	6	94	75	100	94	100	100		
Black Mesdag	15	2	0	100	100				
Miami	0	63	13	100	87			100	100
	3	13	0	100	90	100	100		
	6	75	59	100	100				
	9	93	95	100	100				
Victory	0	0	0	92	86			100	100
Aurora	6	0	0	57	29				
Early Champion	0	0	0	64	29	82	39	100	100
	3	11	7	100	100				
	9	76	62	100	100				
Richland	8	0	0	82	0	89	9		
	12	0	0	59	13				
	18	48	8					100	100
	21	96	70			100	100		
A. fatua, "Allentown"	8	0	0	63	0	87	0		
	12	0	0	90	40				
	15	0	0	89	36				
Markton	11	0	0	66	52				
Brunker	18	0	0	90	55			100	100
	24	27	13	97	75				
Fulghum	15			45	13				
A. sterilis var. *ludoviciana*	11	0	0	69	20				
	21	0	0	46	17				
Weighted average [1]	All	43	30	65	49			100	100
Do.[1]	0	33	20	97	91			100	100

[1] Average of the 4 prompt-germinating varieties (Cornellian, Miami, Victory, and Early Champion) that eventually germinated 100 percent.

TABLE 9.—*Days to germinate and moisture content of fresh seed and seed 3 or 4 days old, and moisture content of straw at harvest, of 25 varieties of Avena*

Germination type and oat variety	Time in germinator until germination—				Moisture content		
	Began		Reached 80 percent		Seed		Straw
	Fresh seed	Seed 3 or 4 days old	Fresh seed	Seed 3 or 4 days old	Fresh	3 or 4 days old	
Prompt:	*Days*	*Days*	*Days*	*Days*	*Percent*	*Percent*	*Percent*
Black Mesdag	4	5	6	8	16.3	13.3	70.5
Cornellian	3	3	6	7	12.6	12.0	75.1
Early Champion	6	3	49	6	12.8	11.9	75.3
Fulghum (winter type)	5	2	11	8	11.0	11.6	70.0
Hairy Culberson	3	5	11	8	10.0	11.3	68.7
Liberty Hull-less	2	3	14	3	14.5	13.5	73.4
Miami	3	3	6	6	13.8	11.8	73.7
Victory	6	7	6	35	13.8	13.4	61.8
Winter Turf	5	2	11	8	11.2	11.8	66.7
Slow:							
A. fatua, "Allentown"	4	5	21	8	32.6	16.6	70.7
Aurora	6	6	(1)	(1)	16.0	13.1	74.7
Richland	6	5	[1] 74	70	17.7	13.3	79.0
Storm King	9	6	(1)	49	14.6	10.9	76.2
Very slow:							
Brunker	18	6	47	44	15.6	11.5	74.8
Fulghum (C. I. 3227)	15	14	77	(1)	27.1	15.3	72.9
Lee	15	12	59	53	12.1	9.6	70.2
Markton	8	27	(1)	(1)	13.8	13.2	73.2
Victor	12	9	68	62	17.4	12.0	69.1
White Tartar	27	13	(1)	58	19.8	11.6	73.5
Delayed:							
A. sterilis var. *ludoviciana*	14	12	(1)	(1)	16.4	15.3	73.8
Berger	29	40	(1)	(1)	13.3	11.3	75.0
Coastblack	56	12	(1)	53	15.3	9.4	68.3
Early Joanette	64	56	80	(1)	21.6	12.4	79.6
Nortex	(2)	(2)	(2)	(2)	23.3	13.7	68.4
Victoria	68	66	(1)	(1)	19.1	14.8	72.2

[1] Did not germinate as much as 80 percent at end of test. [2] Did not germinate within 81 days.

Within 3 or 4 days after harvest all but three varieties had less than 15 percent moisture and could be considered as approximately air-dry. Periodic fluctuations in moisture content due to changes in atmospheric humidity after that time were too small to affect appreciably the condition of the seed.

During this short interval after harvest, retardation in germination was decreased in some varieties along with the reduction in moisture content. The behavior of the varieties in the delayed-germination groups, however, shows that drying alone can have only a limited effect in breaking dormancy in oats.

The moisture content of the straw at harvest time varied from 61.8 to 79.6 percent in the different varieties. There appeared to be little or no relation between promptness of germination and the moisture content of the straw.

SUMMARY

Freshly harvested seed of varieties of *Avena sativa* showed all degrees of prompt, slow, and delayed germination. The freshly harvested seed of the varieties of *A. byzantina*, *A. fatua*, and *A. sterilis* showed slow or delayed germination. A single variety of *A. nuda* germinated promptly. Indications are that dormancy may prove valuable in tracing the probable origin of oat varieties.

After storage for 7 to 10 weeks after harvest, when the experiment had ended, all varieties except Fulghum, Victoria, and *Avena sterilis* var. *ludoviciana* showed a high 7-day germination. Dormancy was no longer evident in many of the varieties after storage for 4 weeks. No Nortex seed stored less than 66 days germinated satisfactorily, thus explaining the poor field stands frequently obtained following the use of freshly harvested seed of Red Rustproof strains.

Delayed germination was not definitely associated with time of maturity, growth habit, or cold resistance. Freshly harvested seed of some varieties contained considerable moisture and did not germinate so rapidly as seed that was allowed to dry for 3 or 4 days. However, many of the varieties continued to germinate slowly after the seed was air-dry, and delayed germination apparently resulted largely from factors other than moisture content of the seed.

Varieties having dark-colored kernels, prominent basal scars, and numerous basal hairs on the primary kernels were slow in germination. These characters are common to varieties belonging to *Avena fatua*, *A. sterilis*, and *A. byzantina*. However, their relationship to delay in germination may be merely incidental.

The varieties that germinated promptly had kernels of medium size. When freshly harvested the primary kernels germinate more promptly than the small and younger secondary kernels of the spikelet. The difference is less evident after a few days' storage.

LITERATURE CITED

(1) COFFMAN, F. A.
1937. SPECIFIC [SPECIES] HYBRIDIZATION, A PROBABLE METHOD FOR PRODUCING HARDIER WINTER OATS. (Note) Jour. Amer. Soc. Agron. 29: 79–81.

(1A) ——— PARKER, J. H., and QUISENBERRY, K. S.
1925. A STUDY OF VARIABILITY IN THE BURT OAT. Jour. Agr. Research 30: 1–64, illus.

(2) DEMING, G. W., and ROBERTSON, D. W.
1933. DORMANCY IN SMALL-GRAIN SEEDS. Colo. Expt. Sta. Tech. Bull. 5 12 pp.
(3) EASTHAM, A.
1928. SOME TECHNICAL ASPECTS OF SEED TESTING. Jour. Natl. Inst. Agr. Bot. 2: 81–84.
(4) ETHERIDGE, W. C.
1916. A CLASSIFICATION OF THE VARIETIES OF CULTIVATED OATS. N. Y. (Cornell) Agr. Expt. Sta. Mem. 10, pp. 79–172, illus.
(5) FOY, N. R.
1932. DELAYED GERMINATION IN CEREALS. New Zeal. Jour. Agr. 44: 129.
(6) GARBER, R. J., and QUISENBERRY, K. S.
1923. DELAYED GERMINATION AND THE ORIGIN OF FALSE WILD OATS. Jour. Heredity 14: 267–274, illus.
(7) HARRINGTON, GEORGE T.
1923. FORCING THE GERMINATION OF FRESHLY HARVESTED WHEAT AND OTHER CEREALS. Jour. Agr. Research 23: 79–100.
(8) HYDE, E. O. C.
1935. OBSERVATIONS ON THE GERMINATION OF NEWLY HARVESTED ALGERIAN OATS. New Zeal. Jour. Agr. 51: 361–367, illus.
(9) JOHNSON, L. V. P.
1935. GENERAL PRELIMINARY STUDIES ON THE PHYSIOLOGY OF DELAYED GERMINATION IN AVENA FATUA. Canad. Jour. Research (C) 13: 283–300.
(10) KINZEL, W.
1900. ÜBER DIE KEIMUNG HALBREIFER UND REIFER SAMEN DER GATTUNG ASCUTA. Landw. Vers. Sta. 54: [123]–133.
(11) LARSON, A. H., HARVEY, R. B., and LARSON, JOHN.
1936. LENGTH OF THE DORMANT PERIOD IN CEREAL SEEDS. Jour. Agr. Research 52: 811–836.
(12) MURPHY, HICKMAN C., and STANTON, T. R.
1930. OAT VARIETIES HIGHLY RESISTANT TO CROWN RUST. (Note) Jour. Amer. Soc. Agron. 22: 573–574.
(13) SIFTON, H. B.
1921. LONGEVITY OF THE SEEDS OF CEREALS, CLOVERS, AND TIMOTHY. Assoc. Off. Seed Analysts North Amer. Proc. 12–13: 22–24.
(14) STANTON, T. R.
1936. SUPERIOR GERM PLASM IN OATS. U. S. Dept. Agr. Yearbook 1936: 347–414, illus.
(14A) STANTON, T. R., and MURPHY, H. C.
1933. OAT VARIETIES HIGHLY RESISTANT TO CROWN RUST AND THEIR PROBABLE AGRONOMIC VALUE. Jour. Amer. Soc. Agron. 25: [674]–683.
(15) WHITCOMB, W. O.
1923. GERMINATION OF NEWLY THRESHED GRAINS. Assoc. Off. Seed Analysts North Amer. Proc. 14–15: 84–89, illus.
(16) ———
1924. DORMANCY OF NEWLY THRESHED GRAIN. Assoc. Off. Seed Analysts North Amer. Proc. 16: 28–34, illus.

TWO DISTINCT STRAINS OF THE NEMATODE APHELENCHOIDES FRAGARIAE OCCURRING ON STRAWBERRY PLANTS IN THE UNITED STATES[1]

By J. R. CHRISTIE [2]

Nematologist, Division of Nematology, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

In the southeastern part of the United States strawberry (*Fragaria* L.) plants showing symptoms of the disease known as dwarf or crimp, harbor in the bud, between the young unfolding leaves, nematodes of the species *Aphelenchoides fragariae* (Ritzema Bos, 1891) Christie, 1932. That this nematode is the cause of the disease has been demonstrated beyond doubt. In Maryland, symptoms usually appear in early July and begin to diminish in severity during October with the advent of cool weather. Farther south the period of pronounced symptoms may be somewhat extended, but even in Florida, according to Brooks,[3] the disease is most pronounced from July through September.

In certain strawberry plantings on Cape Cod near Falmouth, Mass., there occurs a disease somewhat resembling dwarf or crimp of the South. This condition first came to the attention of officials of the Massachusetts Agricultural Experiment Station in 1932. In the buds of plants showing symptoms are harbored nematodes that appear to be morphologically identical with those from southern-grown strawberry plants. There is little reason to doubt that the nematodes are the cause of the trouble. A microscopic examination of diseased plants in itself is very convincing, since the nematodes are usually present in enormous numbers—a much greater population than is found in the most heavily infested southern plants. The period of maximum symptoms is from about the middle of April to the first of June. At other times during the growing season symptoms are much less severe although not necessarily lacking.

Dwarf in the South, therefore, is a midsummer and early fall disease, while the Cape Cod trouble is primarily a disease of early spring. From the standpoint of control measures the reason for this difference in the seasonal occurrence of symptoms is a matter of considerable importance. The question arises as to whether the Cape Cod trouble and southern dwarf are the same disease behaving in a different manner under different climatic conditions or whether they are two distinct diseases. The close morphological similarity of the nematodes infesting strawberry plants in the two regions makes it impos-

[1] Received for publication November 24, 1937; issued July 1, 1938. This investigation was conducted at the Cranberry Substation, East Wareham, Mass., in cooperation with the Massachusetts Agricultural Experiment Station, and at the Coastal Plain Branch Station, Willard, N. C., in cooperation with the North Carolina Department of Agriculture.

[2] At Willard, N. C., the author was materially assisted by G. A. Meckstroth, associate pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[3] BROOKS, A. N. CRIMP—A NEMATODE DISEASE OF STRAWBERRY. Fla. Agr. Expt. Sta. Bull. 235, 27 pp., illus. 1931.

sible for the taxonomists to differentiate two species. However, races or strains, very similar morphologically but differing physiologically, have long been recognized among certain species of plant-infesting nematodes. That they occur in the genus *Aphelenchoides* has already been suggested.[4] Among the forms previously described as distinct species, *A. olesistus* (Ritzema Bos) infests the leaves of begonias, ferns, and other plants; *A. ritzema-bosi* (Schwartz) infests the leaves of chrysanthemums and other plants; *A. subtenuis* (Cobb) is found in narcissus bulbs; and *A. ribes* (Taylor) is a bud parasite of black currants and gooseberries. All these forms are morphologically so similar to the bud nematode of strawberry plants that Steiner and Buhrer[5] regard them as physiological strains of *A. fragariae*. Hitherto strains have been, for the most part, host strains, i. e., strains more or less specialized to certain plants or groups of plants. The writer is aware of no recorded instance where two distinct strains of a nematode species are confined to a single host plant.

The object of the experiments reported in this paper was primarily to determine the possible effect of climate and other regional conditions on the character and seasonal occurrence of disease symptoms in strawberry plants caused by the nematode *Aphelenchoides fragariae*. Plants infested with this nematode from Cape Cod and plants infested with this nematode from North Carolina were grown side by side at Wareham, Mass., and at Willard, N. C. In every instance where symptoms developed they were characteristic of the region from which the nematodes originated and not of the region in which the plants were grown.

For convenience and brevity the terms "spring dwarf" and "summer dwarf" are proposed for the Cape Cod type of disease and the southern type of disease, respectively. For descriptions and figures of summer dwarf the reader is referred to papers by Plakidas,[6] Brooks,[7] and Christie and Stevens.[8] The last-mentioned paper also describes and figures spring dwarf as it occurs on Cape Cod.

EXPERIMENTAL PROCEDURE

There was constructed at Wareham, Mass., a concrete isolation frame (fig. 1) the walls of which extended 18 inches below and 18 inches above soil level and which was of such size that two transverse walls divided it into three compartments, each 6 by 12 feet. A similar concrete isolation frame was constructed at Willard, N. C. The arrangement and treatment of the plants were the same in both the Wareham and Willard experiments, and the following explanation applies in each case. In compartment *A*, fig. 2, Nos. 1 to 16 were strawberry plants of the Howard 17 (Premier) variety, selected in fields near Falmouth as showing typical symptoms of spring dwarf,

[4] CHRISTIE, J. R., and CROSSMAN, LOUISE. WATER TEMPERATURES LETHAL TO BEGONIA, CHRYSANTHEMUM AND STRAWBERRY "STRAINS" OF THE NEMATODE APHELENCHOIDES FRAGARIAE (ANGUILLULINIDAE). Helminthol. Soc. Wash. Proc. 2: 98–103, illus. 1935.

[5] The first three species mentioned were synonymized in the following publication: STEINER, G., and BUHRER, EDNA M. NONSPECIFICITY OF BROWN-RING SYMPTOMS ON NARCISSUS ATTACKED BY NEMATODES. Phytopathology 22: 927–928, illus. 1932. The last species mentioned (*A. ribes*) was synonymized in: STEINER, G. GOOSEBERRY PLANTS AND LILIES ATTACKED BY THE STRAWBERRY NEMATODE, APHELENCHOIDES FRAGARIAE (ANGUILLULINIDAE). Helminthol. Soc. Wash. Proc. 1: 58–59, illus. 1934.

[6] PLAKIDAS, A. G. STRAWBERRY DWARF. Phytopathology 18: 439–444, illus. 1928.

[7] BROOKS, A. N. See footnote 3.

[8] CHRISTIE, J. R., and STEVENS, NEIL E. STRAWBERRY DWARF. U. S. Dept. Agr. Cir. 297, 8 pp., illus. 1933.

and transplanted into the isolation frame; Nos. 17 to 32 were supposedly disease-free plants of the Blakemore variety from Beltsville, Md., set in the isolation frame and subsequently infested with nematodes from diseased Falmouth plants by dropping the parasites, suspended in water, into the crown of the plant with a dropper.

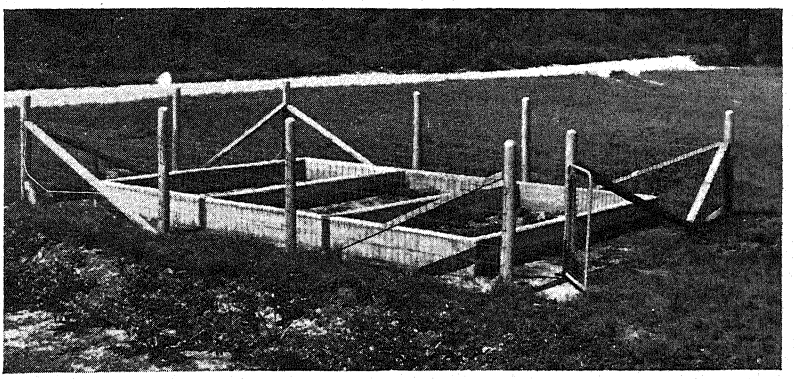


FIGURE 1.—Isolation frame at Wareham, Mass. That at Willard, N. C., was similar in all essential respects.

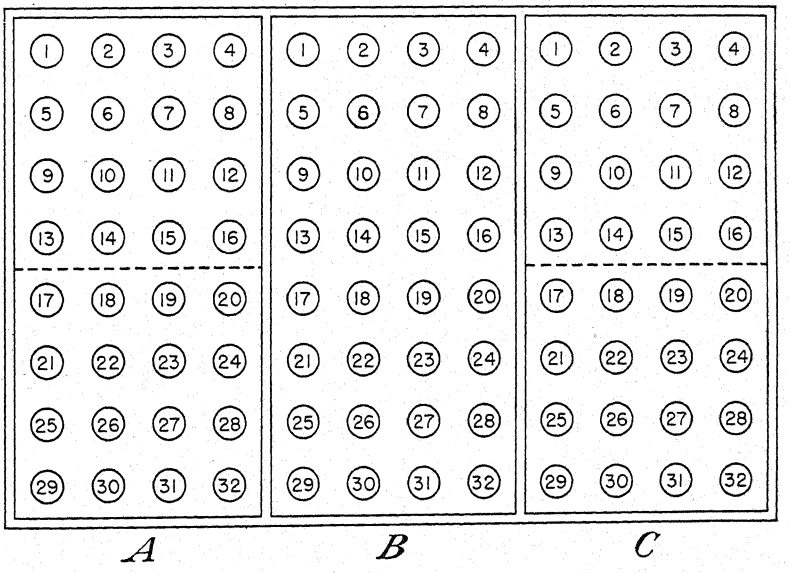


FIGURE 2.—Arrangement of plants in isolation frame at Wareham, Mass., and at Willard, N. C. *A*, 1–16, Plants affected with the Cape Cod disease when selected from fields near Falmouth, Mass., and 17–32, plants experimentally infested with *Aphelenchoides fragariae* from Cape Cod plants; *B*, uninfested controls; *C*, 1–16, plants affected with southern dwarf when selected in fields near Willard, N. C., and 17–32, plants experimentally infested with *Aphelenchoides fragariae* from North Carolina plants.

Compartment *B* was filled with supposedly disease-free plants of the Blakemore variety from Beltsville, to serve as controls.

In compartment *C*, Nos. 1 to 16 were plants of the Blakemore variety selected in fields near Willard, as showing typical symptoms of summer dwarf, and transplanted into the isolation frame. Nos. 17

to 32 were supposedly disease-free plants of the Blakemore variety from Beltsville infested with nematodes from diseased North Carolina plants. In all the compartments a limited number of runner plants were allowed to remain, but enough were removed to prevent the formation of a dense stand. A microscopic examination of runner plants removed in thinning indicated to some extent the degree of infestation at different times of the year. Where runner plants were not formed, a portion of an original plant was sometimes broken away and examined. The Wareham experiment was terminated on August 7, 1936, and the Willard experiment on March 20, 1937. At these times an extensive microscopic examination of plants was made. The situation did not permit keeping these experiments under constant observation. Data were secured during periodic visits to the two localities.

THE WAREHAM EXPERIMENT

Plants 1 to 16, compartment *A*, were set into the isolation frame June 28, 1934. Distinct sypmtoms of spring dwarf appeared on six of the plants during May 1935 but were not as pronounced as when the plants were selected for transplanting the preceding year. None of the plants showed distinct symptoms during the spring of 1936. When the experiment was terminated August 7, 1936, a miscrosopic examination showed an occasional specimen of *Aphelenchoides fragariae* in some of the plants. In this locality, however, plants affected with spring dwarf rarely harbor a large population during late summer. Throughout the course of the experiment these plants remained stunted. Plant 11 set one small runner plant; otherwise runners were not formed.

Plants 17 to 32, compartment *A*, were set into the isolation frame June 25, 1934, and infested 2 days later. So far as could be determined this attempt to establish an infestation was not successful, perhaps owing to subsequent wilting of the plants during midday from the effects of transplanting. A second attempt to establish an infestation was made May 24, 1935, and was successful, at least for some plants, although clearly recognizable symptoms never developed. An occasional specimen of *Aphelenchoides fragariae* was found in some of the plants on microscopic examination when the experiment was terminated August 7, 1936.

Plants 1 to 16, compartment *C*, were set into the isolation frame July 12, 1934. Symptoms of summer dwarf were retained throughout the summer but decreased somewhat in severity following transplanting and largely disappeared during September. On September 20, one runner plant from each of eight original plants was removed and examined. Of these, two had a moderately heavy infestation, three harbored a few specimens, and three were not infested. No indication of symptoms appeared during the spring and summer of 1935 or 1936. All the original plants that were still alive and most of the runner plants were carefully examined August 7, 1936, and not a specimen of *Aphelenchoides fragariae* was found.

Plants 17 to 32, compartment *C*, were set into the isolation frame June 25, 1934, and infested July 13. On September 20, of the original plants, six showed summer dwarf symptoms of varying degrees of severity and two were dead. The most pronounced symptoms were on the runner plants, many of them resembling in every respect

similarly affected plants in the South. One runner plant from each of 12 of the original plants was examined; 7 had a moderately heavy infestation, 2 harbored a few specimens, and 3 were not infested. Of these plants some of the most heavily infested showed moderate symptoms, the others were not noticeably abnormal. No indication of symptoms appeared during the seasons of 1935 and 1936. On August 7, 1936, a careful examination of all the remaining original plants and many of the runner plants failed to disclose the presence of *Aphelenchoides fragariae.*

None of the uninfested plants in compartment *B* developed recognizable symptoms. From time to time throughout the duration of the experiment, runner plants were removed and examined. All plants whose appearance could in any way be regarded with suspicion were included among those examined, but *Aphelenchoides fragariae* was never found.

THE WILLARD EXPERIMENT

Plants 1 to 16, compartment *A*, were set into the isolation frame May 29, 1935. They were shaded with burlap for a few weeks, then covered with slat frames providing one-third shade until September 14, when all shade was permanently removed. Since diseased Blakemore plants were not available in the Falmouth region, it was necessary to use Howard 17 plants, a variety that does not thrive in the southern climate. Furthermore, these plants could not be selected until considerably past the time when transplanting can be done most successfully at Willard. Partial shade during the hottest part of the first summer seemed advisable. Many of these plants never entirely lost their abnormal appearance during the summer of 1935, although such symptoms as persisted were much less pronounced than when the plants were selected for transplanting. On January 16, 1936, three of these plants showed quite pronounced symptoms resembling infested plants on Cape Cod during May, and several others showed slight but recognizable spring dwarf symptoms. A part of the crown of two of the plants showing slight symptoms was removed for examination; each harbored a heavy population of *Aphelenchoides fragariae,* characteristic of spring dwarf. During the summer of 1936, as during the preceding summer, some of these plants never entirely lost their abnormal appearance although such symptoms as persisted were much less pronounced than during early spring. In 1937, observations were made on March 20, when the experiment was terminated and the plants were examined. All plants were small and had a stunted appearance. One showed pronounced symptoms and was heavily infested. Eight showed slight but recognizable symptoms; of these, three harbored a heavy infestation, five a moderately heavy infestation, and one a few specimens. Of the remaining plants, four did not show symptoms and were not infested and three were dead.

Plants 17 to 32, compartment *A*, were set into the isolation frame December 7, 1934, and infested May 29, 1935. These plants were shaded in the same manner and for the same period as Nos. 1 to 16. All plants were apparently normal on July 18, 1935, but by August 13, three had developed suspiciously crinkled leaves and one showed distinct symptoms. By September 14 these abnormalities had dis-

appeared. On January 16, 1936, four plants showed moderately pronounced spring dwarf symptoms and several others showed slight but recognizable symptoms. In some cases traces of these abnormalities persisted throughout the summer. On March 20, 1937, seven plants showed recognizable symptoms and all were heavily infested; three plants did not show symptoms and were not infested; and six plants were dead.

Plants 1 to 16, compartment *C*, were set into the isolation frame July 6, 1934. Distinct symptoms reappeared on nine of the plants during July 1935 but were less pronounced than when the plants were selected for transplanting the previous year. Recognizable symptoms appeared on some of the plants during August and September 1936 but were less pronounced than in 1935. Very few runners were produced at any time, although the original plants became large with many subdivisions of the crown. On March 20, 1937, a microscopic examination revealed only an occasional specimen of *Aphelenchoides fragariae* on some of the plants. This was in agreement with the nematode population usually found in infested southern plants at this time of the year but was in marked contrast to the very heavy infestation encountered in plants 1 to 16, compartment *A*, infested with the Cape Cod strain.

Plants 17 to 32, compartment *C*, were set into the isolation frame December 7, 1934, and infested July 19, 1935. Ten of the plants had developed typical symptoms by September 14, 1935. Symptoms were also conspicuous on many of the runner plants, of which a greater number had been set than was the case with plants 1 to 16. During midsummer of 1936 recognizable symptoms appeared on some of the plants, but they were less pronounced than during the preceding summer. When the plants were examined microscopically on March 20, 1937, a specimen or two of *Aphelenchoides fragariae* were found on some of them by careful searching.

The plants in compartment *B* were set into the isolation frame December 7, 1934. No indication of symptoms was seen during 1935. From time to time runner plants and any plants that seemed abnormal in appearance were removed for examination, but all were free from the parasite. Late in the summer of 1936 two plants developed symptoms of summer dwarf, and microscopic examination showed that both were infested with *Aphelenchoides fragariae*. The origin of this infestation is not known.

DISCUSSION

Diseased strawberry plants from Cape Cod, when grown at Wareham, Mass. (plants 1–16, compartment *A*) and diseased strawberry plants from North Carolina, when grown at Willard, N. C. (plants 1–16, compartment *C*), showed most severe symptoms during the first year and progressively less severe symptoms thereafter. In general, both lots produced few runners. The plants at Wareham experimentally infested with the Cape Cod strain (plants 17–32, compartment *A*) were little affected by the parasite, and their growth throughout the experiment was about the same as that of the controls. For some reason the experimental infestation did not result in a sufficient nematode population to materially affect the plants. The plants at Willard experimentally infested with the southern strain (plants

17–32, compartment *C*) produced runners rather freely the first year but thereafter produced fewer runners than did the controls.

Diseased southern plants grown at Wareham (plants 1–16, compartment *C*) produced few runners during the experiment even though the nematode population gradually disappeared. Plants at Wareham experimentally infested with the southern strain (plants 17–32, compartment *C*) produced runners rather freely throughout the experiment. During the first year many of these runner plants showed typical and quite pronounced symptoms of dwarf; but these symptoms did not reappear during the following two summers, apparently because of the gradual reduction of the nematode population that was unable to maintain itself under northern conditions.

The behavior of diseased Cape Cod plants grown at Willard (plants 1–16, compartment *A*) and of plants experimentally infested with the Cape Cod strain (plants 17–32, compartment *A*) was about the same. Symptoms were clearly recognizable in 1936 as early as January 3, when the plants harbored the heavy nematode population characteristic of infested plants during April and May in the vicinity of Falmouth. It should be recalled in this connection that from January to March plants affected with summer dwarf and growing under southern field conditions never develop pronounced symptoms or harbor a large nematode population.

During January, February, and March, 1931, the number of *Aphelenchoides fragariae* harbored by each of 65 plants was determined. These plants were of the variety Klondike, selected in fields near Chadbourn, N. C., during the summer of 1930 when all showed pronounced symptoms. Counts were made on five plants each week. The greatest number of *Aphelenchoides fragariae* specimens found in any plant was 80, and the average for the 3 months was 10 per plant. Counts on 5 plants per week, or a total of 20 plants, from the same locality during September 1931 gave an average of 221 specimens per plant, the greatest number in any one plant being 605. Five plants selected in a field near Falmouth on April 24, 1934, and showing typical symptoms of spring dwarf harbored approximately 2,800, 8,620, 7,800, 9,700, and 12,900 specimens of *Aphelenchoides fragariae*, respectively.[9]

The intensity of the nematode infestation and the time of the year when it becomes greatest are the most outstanding differences between spring dwarf and summer dwarf. The plants in compartment *A* at Willard affected with spring dwarf retained the tendency to harbor a large population of *Aphelenchoides fragariae* during early spring, when plants affected with summer dwarf have only a few specimens. During January 1936 and March 1937, many of the infested plants in compartment *A* had a much greater nematode population than is found in infested North Carolina plants during the period of maximum symptoms.

These experiments indicate that the differences between the behavior of the parasite causing summer dwarf and that causing spring dwarf and the respective seasonal occurrence of symptoms are not due to the influence of climate or other regional conditions. All the facts indicate that two diseases are being dealt with which result from two distinct races or strains of *Aphelenchoides fragariae*. The

[9] CHRISTIE, J. R., and BOYD, O. C. APHELENCHOIDES FRAGARIAE ON CAPE COD STRAWBERRIES. U. S. Bur. Plant Indus. Plant Disease Reptr. 18: 45–46. 1934. [Mimeographed.]

potential geographic range of each disease, however, may be restricted by climatic conditions. That summer dwarf can become permanently established as far north as Massachusetts seems doubtful. Spring dwarf occurs in the regions of Falmouth and Dighton, Mass., but has not been found elsewhere in the New England States. What is in all probability the same disease has been found near Merchantville, N. J., on the Eastern Shore of Maryland, and in Fairfax County, Va. From the results of the Willard experiment one would conclude that this disease probably can and perhaps does occur as far south as North Carolina.

SUMMARY

When strawberry plants showing typical symptoms of summer dwarf were moved from North Carolina to Wareham, Mass., they retained, to some extent, both their symptoms and their nematode population throughout the remainder of the growing season. When strawberry plants growing at Wareham were experimentally infested during early summer with *Aphelenchoides fragariae* from southern plants, typical symptoms of summer dwarf were freely developed during July and August. In neither case were recognizable symptoms developed during the following two summers, and at the end of the third season the plants had entirely lost their infestation. There was no tendency for northern climatic conditions to change the seasonal occurrence of symptoms; but instead, symptoms disappeared after the first summer, apparently owing to the gradual extermination of the nematodes.

When strawberry plants showing typical symptoms of spring dwarf were moved from Falmouth, Mass., to Willard, N. C., neither the character nor the seasonal occurrence of the disease was changed except that symptoms occurred much earlier in the year. Plants growing at Willard and experimentally infested with nematodes from Cape Cod plants behaved in a similar manner. After two summers in the southern climate there was no significant reduction in the nematode population.

It is concluded that spring dwarf and summer dwarf are caused by two different strains of *Aphelenchoides fragariae* that are indistinguishable morphologically but differ physiologically.

U. S. GOVERNMENT PRINTING OFFICE: 1938

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 57 WASHINGTON, D.C., JULY 15, 1938 No. 2

PRESERVATION OF CYANOGENETIC PLANTS FOR CHEMICAL ANALYSIS [1]

By REINHOLD R. BRIESE, *junior chemist*, and JAMES F. COUCH, *chemist, Pathological Division, Bureau of Animal Industry, United States Department of Agriculture*

INTRODUCTION

The need of an efficient preservative for samples of fresh cyanogenetic plants intended for chemical analysis has long been recognized. In the past, reliable figures for the hydrocyanic acid content of such plants could be obtained only from specimens collected immediately before analysis. This fact is due to the rapidity with which the hydrocyanic acid content changes after the plants are gathered, resulting in a variable loss of hydrocyanic acid which renders the analytical figures uncertain. Considerable disappearance of hydrocyanic acid occurring when cyanogenetic plants are stored with water has been reported by Dezani, (*13*) [2] Alsberg and Black, (*3*) and others (*20*, *26*). These considerations make impossible a comparison of plants grown in different places under different climatic and soil conditions if the samples must be transported great distances for analysis.

In a projected study of the factors involved in cyanogenesis it was desired to obtain samples of several varieties of cyanogenetic plants from widely scattered regions. These included localities in Utah, Colorado, Oklahoma, Texas, and Virginia. The chemical studies were conducted in the laboratories of the Bureau of Animal Industry in Washington, D. C. The transportation of some of the samples required 7 days by express, so that a long interval elapsed between collection and analysis. In some cases it appeared that refrigeration would preserve the samples without great change during transit, but this method was impracticable. It was decided, therefore, to study other means as well as refrigeration for preservation of the samples, and this paper records the results of that investigation.

FORMATION AND PRESERVATION OF HYDROCYANIC ACID IN PLANTS

Hydrocyanic acid does not appear to exist as such in cyanogenetic plants (*9*, *17*, *18*, *21*) but results from the interaction of an enzyme ("emulsin") and a glucoside which contains a CN group capable of being hydrolyzed to hydrocyanic acid. The rate of hydrocyanic acid formation, once the process begins, is originally rapid but diminishes after some hours and then proceeds slowly for an indeterminate time. Studies by Auld (*4*, *5*), Caldwell and Courtauld (*8*), Tammann (*24*), and Willstätter and Csányi (*31*) indicate that the enzymic hydrolysis of amygdalin is not complete. Auld observed a decomposition of 93.3 percent in 22 hours, and Caldwell and Courtauld report 98.2 and 98.5

[1] Received for publication March 8, 1938; issued August 1938.

[2] Italic numbers in parentheses refer to Literature Cited, p. 105.

percent in 67 and 90 hours, respectively. Auld considers these percentages too high.

Charlton (*9*) and, independently, Warth (*28*) found that after enzymic hydrolysis of Burma beans a further quantity of hydrocyanic acid could be obtained by extraction with hot water and acid hydrolysis of the extract. Narasimha Acharya (*1*) states that 72 hours is required for complete cyanogenesis in sorghum. Under these conditions it is essential that a preservative should not inactivate the enzyme present in the plant and so stop all cyanogenesis, and in addition that it should so act as to prevent or at least minimize loss of the hydrocyanic acid liberated by enzyme action. The ideal preservative would be one that could act solely to prevent loss of hydrocyanic acid without disturbing other metabolic processes in the sample, and that would permit quantitative recovery of the hydrocyanic acid produced during the preservation.

Some attempts to obtain an effective preservative have been reported. Brünnich (*7*) stated that the addition of formalin or chloroform to sorghum completely stops formation of hydrocyanic acid. Alsberg and Black (*2*) employed alcohol to preserve specimens of certain grasses. Willaman and West (*30*) used 20 cc of 3-percent alcoholic sodium hydroxide and 2 cc of chloroform for samples preserved for 4 to 8 days and reported no loss of hydrocyanic acid in the material. Willaman (*29*) in a later study found alcohol and mixtures of alcoholic sodium hydroxide with chloroform and of alcohol with chloroform or ether to be effective preservatives of sorghum. He concluded that alcohol, chloroform, and ether stimulate the synthetic action of the glucoside enzymes, leading to an increased hydrocyanic acid content of the plant.

BACTERIAL DECOMPOSITION

In the storage of plant material the question of the possibility and effect of bacterial decomposition arises. The observation that certain micro-organisms may so decompose protein as to lead to the formation of hydrocyanic acid introduces a factor that must be considered in all studies on preservation. Emerson, Cady, and Bailey (*14*) report that certain micro-organisms living on slightly acid protein media evolve hydrocyanic acid provided no free mineral acid is present. Clawson and Young (*10*) identified one of these organisms as *Bacillus pyocyaneus* and stated that *B. fluorescens* and other organisms produce hydrocyanic acid from proteins. Clawson and Young call attention to the fact that in most of the work that has been done on cyanogenetic plants the workers were using nonsterile material "which could have been easily contaminated by a hydrocyanic acid-producing organism."

The probability that this explanation of cyanogenesis would account for the hydrocyanic acid content reported for *Phaseolus lunatus*, sorghum, wild cherry, flax, and other cyanogenetic plants was removed by the isolation of the cyanophoric glucosides present in these plants and the proof that they may evolve hydrocyanic acid under sterile conditions such as boiling with strong mineral acids.

Further evidence on this point is afforded by the present study in which it has been found that cyanogenetic plants preserved with 1 and 2 percent of mercuric chloride evolved as much and in some cases more hydrocyanic acid than control plants stored under nonsterile conditions.

PLANTS USED IN THIS INVESTIGATION

A number of different species of cyanogenetic plants were used in this study since it was not known whether an effective preservative for one type of plant would be equally effective for a different species. In most cases both fresh and dried material was available, but for Johnson grass (*Sorghum halepense*) only dried plant was obtained. Wild cherry (*Prunus serotina*) was collected in Arlington County, Va., as needed. Another species, *P. melanocarpa*, was collected in the neighborhood of Salina, Utah, by A. B. Clawson, of the Bureau of Animal Industry, and shipped to the laboratory both as dried plant and in preservative. Arrowgrass (*Triglochin maritima*) was also collected by Clawson in various localities in Utah and was shipped both as dried plant and in preservative. Sudan grass (*S. vulgare* var. *sudanensis*) was collected near Denver, Colo., by G. W. Stiles, Jr., of this Bureau, and was shipped both as green and as dried plant. Dried Johnson grass was obtained from Woodward, Okla., through the Bureau of Dairy Industry. A large number of specimens of sorghum (*S. vulgare*) were obtained from a variety of sources. Through the courtesy of J. H. Martin, of the Bureau of Plant Industry, growing plants of 15 varieties of sorghum were made available, and later plants growing under greenhouse conditions were provided. Dried material of 12 varieties of sorghum grown at Chillicothe, Tex., was obtained through the courtesy of J. C. Stephens, of the Bureau of Plant Industry. The principal studies of sorghum were made with the varieties hegari, spur feterita, and white Italian broomcorn. Specimens of green arrowgrass and *P. melanocarpa* were preserved by Clawson in Utah before shipment. All other specimens used in this study were preserved in the laboratory at Washington, D. C.

METHOD OF PREPARING AND PRESERVING SPECIMENS

Customarily 100 g of fresh plant was taken. The quantity of dried plant used was 10, 25, or 50 g, depending on the cyanide content of the specimen. The dried plant was ground through a food chopper or mill, placed in a pint-sized clamp-topped preservative jar equipped with a glass top and rubber gasket, the preservative was added, and, except in special cases, the jar was filled to the shoulder with distilled water. Tap water of high quality was used in cases where no interference could be caused by the dissolved matter.

In the preparation of the samples of fresh plant used in these experiments, the material was passed through a food chopper equipped with the rotary cutter of 1-cm orifice measured at the circumference. The hash was then thoroughly mixed, any juice that had been squeezed out being added, a portion was taken for moisture determination, and the remainder was weighed into sample jars as rapidly as possible.

The specimens preserved at Washington were then stored at definite temperatures until required for use. Since field specimens could be received at this laboratory within 7 days after collection it was accepted, at first, that a preservative that would prevent undue change in the specimen for that length of time would be suitable for use. Consequently most of the early experimental samples were not kept for longer periods. However, when it was found that some substances seemed to possess very good preservative powers a number of experi-

ments were conducted to determine the length of time specimens might be stored and still yield accurate figures on analysis.

METHOD OF ANALYZING SPECIMENS FOR HYDROCYANIC CONTENT

The hydrocyanic acid content of the various samples was determined by the method of Liebig as modified by Denigès (*12*). No difficulty that could be traced to the plant alone was experienced, but on several occasions the preservative rendered the end point obscure or titration quite impossible. The plant specimens were customarily washed into a 5-liter flask with enough water to make 1,200 to 1,500 cc of final volume, and the mixture was distilled over a free flame into 10 cc of 5-percent potassium hydroxide solution. Approximately 400 cc of distillate was taken and titrated, after which distillation was continued until only insignificant quantities of cyanide collected in the receiver.

SOURCES OF ERROR

The principal source of error in this study originated in the method of sampling the green plant. It was impossible to obtain a homogeneous mixture from which several samples might be taken for comparative analyses without allowing considerable loss of hydrocyanic acid and so rendering the results worthless. Since the distribution of cyanogenetic substances is not uniform in various types of tissues in the plant, any excess of one type will alter the hydrocyanic acid content of the sample. Even in samples restricted to leaves there is a difference in the various portions of the leaf structure (*25*), and with samples that contain a portion of stalk, which is fibrous and difficult to comminute and distribute uniformly through the ground sample, the probability of error is much increased. Dried material may be ground to fine powder and intimately mixed so that error from this source is much diminished but not completely eliminated.

Another source of possible error arose in the transference of the stored sample to the flask in which it was to be distilled. With warm samples from the incubator at 37° C., especially when the hydrocyanic acid content is high, there is a possibility that hydrocyanic acid vapor may escape while the sample is being transferred. In cases in which this loss was likely to be appreciable, the samples were weighed into the distilling flask and the preservative solution was added at 37° if the sample was to be incubated. The flask was then tightly stoppered and was not opened until it was connected to the condenser for the actual distillation.

The probable loss of hydrocyanic acid by reaction in the contents of the flask during distillation appears to be a source of error inherent in the process. Aside from losses of hydrocyanic acid due to incomplete cyanogenesis and disappearance of formed hydrocyanic acid by processes in the stored sample, the error inherent in the method may reach 1 percent. Since precision of the order attained in ordinary inorganic analytical procedure is not required in studies of the type reported in this paper, an error of this magnitude is small as compared with experimental differences and does not invalidate the conclusions to be drawn from the experiments. Much more serious are the errors derived from incomplete cyanogenesis and from loss of hydrocyanic acid by apparent conversion into other substances.

PRESERVATIVE ACTION OF DIFFERENT SUBSTANCES

Since the absolute hydrocyanic acid content of the plants under investigation was not known and the figures obtained by various experimental treatments differed within wide limits, it was necessary arbitrarily to select one of the results as a standard with which the other figures and processes might be compared. Ordinarily the highest figure obtained was selected as the standard. It represents the figure that the chemist would report as the hydrocyanic acid content of the sample.

It was found convenient to calculate the analytical results in milligrams of hydrocyanic acid per 100 g of plant rather than in percent. Consequently all the tabular data are expressed in that unit. Figures for green plant are reported on a green rather than on a dry basis since no moisture determinations are available for plants collected at a distance and shipped in preservatives.

UNTREATED SAMPLES

Specimens of the plants were ground through a food chopper, and the weighed quantity was placed in the preserving jar. The specimens were then stored at some definite temperature for the stated time and analyzed. The results (table 1) showed that hydrocyanic acid disappeared during storage in larger than permissible quantities when the specimens were stored at temperatures likely to be encountered during shipment from the place of collection to the laboratory. As indicated by table 1, hegari kept at a low temperature suffered no loss in 4 days.

TABLE 1.—*Hydrocyanic acid recovered from fresh plants after storage without added preservative for different periods*

Plant	Period of preservation	Temperature of preservation	Hydrocyanic acid per 100 g of plant in—		Hydrocyanic acid recovered
			Standard	Test specimen	
	Days	*°C.*	*Milligrams*	*Milligrams*	*Percent*
Arrowgrass	6	(1)	163.8	136.8	83.5
Hegari	1	37	16.5	10.5	63.6
Do	4	9	7.56	7.56	100
Prunus serotina	3	25	131.8	114.4	86.8
Do	6	25	131.8	109	82.7
Sudan grass	3	25	116.6	19.4	16.6

[1] Temperature was variable owing to the transport of the samples across country in summer weather.

REFRIGERATION

The observation that hegari apparently did not change after storage for 4 days at 9° C. suggested further study of this means of preservation. A collection of fresh Spur feterita leaves was minced in a food chopper, and 100-g samples were weighted into 5-liter flasks. To one of these, 1,200 cc of water was added and the hydrocyanic acid was distilled. The sample gave 14 mg of hydrocyanic acid per 100 g of plant. A second sample was mixed with 1,200 cc of water, incubated 24 hours at 37°, and then gave 26.5 mg of hydrocyanic acid per 100 g. This figure was taken as the standard. A third sample

was allowed to remain without the addition of any other substance for 36 hours at laboratory temperature (25°) and then analyzed. It gave 3 mg per 100 g. The other samples were put in two refrigerators one at 9° and the other at 10°. After 24 hours, five of the samples in the refrigerator at 9° were removed, one was mixed with 1,200 cc of water and distilled, and the remaining four were placed in a cold-storage chamber at −10°. The refrigerated samples were withdrawn at 24-hour intervals beginning at 48-hour intervals, mixed with 1,200 cc of water, and the hydrocyanic acid distilled. The results are reported in table 2. None of the refrigerated samples gave as much hydrocyanic acid as the standard, the quantity ranging from 46 to 66.2 percent of the criterion. To determine whether this result was due to suspension of cyanogenesis during refrigeration, one sample was incubated at 37° with 1,200 cc of water for 24 hours. It yielded only 4.05 mg, or 15.3 percent, of the standard. The experiment showed that the mechanism by which hydrocyanic acid is destroyed was considerably increased by the treatment. The results indicated that refrigeration as a method of preservation would not lead to accurate results so far as sorghums are concerned.

TABLE 2.—*Recovery of hydrocyanic acid from fresh Spur feterita leaves after refrigeration under varying conditions*

Period of preservation and temperature (° C.)	Hydrocyanic acid in 100 g of test specimen	Hydrocyanic acid recovery	Period of preservation and temperature (° C.)	Hydrocyanic acid in 100 g of test specimen	Hydrocyanic acid recovery
	Milligrams	*Percent*		*Milligrams*	*Percent*
None	14	52.8	24 hours at 9° then 48 hours at −10°; 24 hours at 37°	4.05	15.3
24 hours at 37° [1]	26.5	100.0	24 hours at 9° then 72 hours at −10°	15.7	59.2
36 hours at 25°	3	11.3	24 hours at 9° then 96 hours at −10°	15.77	59.5
24 hours at 9°	15.5	58.5			
24 hours at 10°	12.2	46.0			
48 hours at 9°	15.5	58.5			
96 hours at 9°	17.55	66.2			
120 hours at 9°	12.69	47.9			
24 hours at 9° then 48 hours at −10°	15.5	58.5			

[1] Taken as the standard.

WATER ALONE

A few specimens were stored with water as the only added substance, with the results shown in table 3.

TABLE 3.—*Hydrocyanic acid recovered from fresh plants after storage in water for 3 or 6 days*

Plant	Period of preservation	Temperature of preservation	Hydrocyanic acid per 100 g of plant in—		Hydrocyanic acid recovered
			Standard	Test specimen	
	Days	*° C.*	*Milligrams*	*Milligrams*	*Percent*
Arrowgrass	6	(1)	163.8	163.8	100
Prunus serotina	3	25	131.8	123	93.3
Sudan grass	3	25	116.6	96.2	82.5

[1] See footnote 1, table 1.

CHLOROFORM MIXTURES

A number of experiments were made with chloroform alone and mixed with water or alcoholic potassium hydroxide (3 percent), or both. In one experiment the alkali was replaced by tartaric acid, which showed a high degree of preservation. In this test 100 g of ground hegari was mixed with 300 cc of water containing 5 g of tartaric acid and 2 cc of chloroform, incubated 24 hours at 37°, and then stored for 17 hours at 9°. The mixture gave 7.38 mg of hydrocyanic acid per 100 g of plant as against 7.56 mg in the standard, or a recovery of 97.6 percent. In one experiment, as shown in table 4, a larger yield of hydrocyanic acid was obtained after 4 days of preservation of hegari with chloroform alone than after 1 day of incubation with water alone, the figure for which is taken as standard. This result may be explained by assuming that chloroform depressed the hydrolysis of the hydrocyanic acid during the time of storage. On the whole the mixtures used cannot be considered satisfactory preservatives, and in addition the presence of chloroform in the distillates during analysis caused a milkiness at times that interfered with observation of the end point.

TABLE 4.—*Hydrocyanic acid recovered from fresh plants after storage with chloroform mixtures for different periods*

Composition of preservative			Plant	Period of preservation	Temperature of preservation	Hydrocyanic acid per 100 g of plant in—		Hydrocyanic acid recovered
Chloroform	Alcoholic potassium hydroxide	Water				Standard	Test specimen	
Cc	*Cc*	*Cc*		*Days*	*°C.*	*Milligrams*	*Milligrams*	*Percent*
2			Arrowgrass	6	(1)	163.8	139.9	85.4
2		300	do	6	(1)	163.8	146	89.1
2			Hegari	4	25	7.56	8.2	108.4
2		300	do	1	9	17.75	17.8	100.0
4			do	1	37	10	9.2	92.0
2	20		do	4	25	7.56	6	79.4
2	20		do	8	25	7.56	6	79.4
2	20	280	*Prunus serotina*	1	25	72.4	23.8	32.9
4			do	1	37	58.9	54.0	91.7
2			do	3	25	131.8	113.4	86.0
2	20	280	Spur feterita	7	25	30.4	10.4	34.2
2		300	Sudan grass	3	25	116.6	96.2	82.5
2		300	White Italian broomcorn	30	25	26.5	21.6	81.5
2		300	do	60	25	26.5	16.2	61.1

[1] See footnote 1, table 1.

ACIDS

Although it is well known that increasing the hydrogen-ion concentration of cyanogenetic mixtures results in a decreased yield of hydrocyanic acid (*11*, *21*, *27*, *29*), it was considered advisable to obtain data on this phenomenon under the conditions of these experiments. Various strengths of different acids in water were added to samples of the plants, and some of the mixtures were then incubated for 24 hours at 37° C. Several of these were then stored in an ice box at 9° for a further 17 hours before analysis.

TABLE 5.—*Hydrocyanic acid recovered from fresh plants after storage with different acids in the dilution indicated for different periods*

Acid	Strength of preservative[1]	Water, as diluent	Plant	Period of preservation	Temperature of preservation	Hydrocyanic acid per 100 g of plant in—		Hydrocyanic acid recovered
						Standard	Test specimen	
	Percent	*Cc*		*Hours*	*°C.*	*Milligrams*	*Milligrams*	*Percent*
Aluminum sulphate[2]	6	1,000	*Prunus serotina*	1	25	116.3	95.2	81.9
Lactic	3	300	Sudan grass	24	37	16.5	15.4	93.3
Do	1	300	do	24	37	16.5	12.7	76.9
Phosphoric	4	300	do	41	[3]	7.56	3.88	51.3
				Days				
Salicylic	1	300	White Italian broomcorn	3	25	26.5	24.8	93.6
Do	1	300	Sudan grass	3	25	116.6	109	93.5
Do	1	300	*P. serotina*	3	25	131.8	127.4	96.7
Do	1	300	do	3	25	131.8	125.3	95.0
Do	5	300	Arrowgrass	6	[4]	163.8	147.7	90.2
				Hours				
Sulphuric	2	300	Sudan grass	41	[3]	7.56	5.68	75.1
Do	3	300	do	24	37	16.5	3.5	21.2
Do	5	300	White Italian broomcorn	1	25	26.35	5.4	20.5
Tartaric	10	300	Hegari	41	[3]	7.56	6.59	87.1
Do	10	300	do.[5]	41	[3]	7.56	6.76	89.4
Do	5	1,000	do.[5]	41	[3]	10	10.8	108.0
Do	5	300	do	24	37	16.5	12.2	73.9

[1] Calculated on weight of plant.
[2] Acid in reaction, though not technically an acid.
[3] 24 hours at 37° and 17 hours at 9°.
[4] See footnote 1, table 1.
[5] Frosted.

The results are reported in table 5, the strength of the acid being calculated to the weight of plant and not to the volume of liquid. Thus, 10 percent of acid refers to 10 g of acid per 100 g of plant used in the experiment. The results obtained from the use of the different acids were in general similar, the differences noted suggesting that the effective agent in loss of hydrocyanic acid is the concentration of hydrogen ions. The greatest effects were obtained with sulphuric and phosphoric acids and less with weak acids, such as lactic and salicylic. Indeed, if salicylic acid did not distill with the hydrocyanic acid and tend to interfere with the subsequent titration it would merit further consideration as a preservative. The effect of lactic acid was of interest because of its possible formation in silage and the findings of Collins (*11*) that lactic acid markedly depresses the rate of evolution of hydrocyanic acid from linseed. The question whether the observed effects of various acids are to be referred solely to hydrogen-ion concentration or whether the character of the anion is also a factor is being investigated in this laboratory.

ALKALIES

A few experiments were made to determine the effect of alkalies on the cyanogenetic plants used in this study. These tests were usually made in connection with experiments on other preservatives and served to furnish comparative data. The results obtained, reported in table 6, are in agreement with those found by Swanson, (*23*) Auld, (*5*) and others. They raise the important question whether the effect of the alkali is inhibition of the cyanogenesis or destruction of the

hydrocyanic acid after its formation. The data, although too few in number to permit conclusions, indicate the probability of the latter alternative. With *Prunus serotina*, in which ordinarily cyanogenesis takes place very rapidly, there was an 80 to 90 percent decrease in hydrocyanic acid recovered, whereas with Sudan grass, in which cyanogenesis is somewhat slower, the decrease was 91 to 96 percent. With alcoholic potassium hydroxide, in which case the alcohol would be expected to repress cyanogenesis and so act to heighten the effect of the alkali if the first alternative were true, actually the contrary effect was noted. Increasing the quantity of alcohol present resulted in a larger yield of hydrocyanic acid.

TABLE 6.—*Hydrocyanic acid recovered from fresh plants after storage in alkaline liquids in the dilutions indicated*

Preservative	Strength of preservative	Diluent [1]	Plant	Period of preservation	Temperature of preservation	Hydrocyanic acid per 100 g of plant in—		Hydrocyanic acid recovered
						Standard	Test specimen	
	Pct.			*Days*	*°C.*	*Milligrams*	*Milligrams*	*Pct.*
Calcium hydroxide	2	Water	Sudan grass	3	25	116.6	10	8.6
Do	5	do	*Prunus serotina*	3	25	131.8	27.0	20.5
Do	10	do	do	3	25	131.8	21.6	16.4
Magnesium oxide	5	do	Arrowgrass	6	(2)	163.8	57.8	35.3
Potassium hydroxide	2	25 per cent alcohol	*P. serotina*	3	25	158.8	14	8.8
Do	2	50 percent alcohol	do	3	25	158.8	21.6	13.6
Sodium carbonate	2	Water	White Italian broomcorn.	3	25	35.6	11.8	33.2
Do	2	do	Sudan grass	3	25	116.6	4.4	3.8
Do	2	do	*P. serotina*	3	25	158.8	14.6	9.2
Do	2	do	Arrowgrass	6	(2)	163.8	112.1	68.4

[1] 300 cc was used in all cases.
[2] See footnote 1, table 1.

MISCELLANEOUS PRESERVATIVES

The results obtained in tests of a number of substances which proved not to be suitable are reported in table 7. Toluene was used because of its well-known antiseptic action. With one sample of hegari it yielded an increased quantity of hydrocyanic acid. However, during the experiment, the mixture was stored at a low temperature, 9° C., a condition under which another sample of hegari without preservative lost no hydrocyanic acid during 4 days' storage (table 1). With samples of other plants the loss of hydrocyanic acid was too large to warrant the further use of toluene which, in addition, has the disadvantage of distilling over with the hydrocyanic acid and interfering somewhat with the titration.

Glucose, thymol, and hexone added to samples of *Prunus serotina* resulted in mixtures that yielded as much hydrocyanic acid after 24 hours as did a sample incubated at the same temperature for 24 hours with the addition of water only. Thymol and hexone distill with the hydrocyanic acid and may interfere with the titration.

TABLE 7.—*Hydrocyanic acid recovered from fresh plants after storage with miscellaneous preservatives in water solution for 1 to 3 days*

Preservative	Strength of preservative	Plant	Period of preservation	Temperature of preservation	Hydrocyanic acid per 100 g of plant in—		Hydrocyanic acid recovered
					Standard	Test specimen	
	Percent		*Days*	*°C.*	*Milligrams*	*Milligrams*	*Percent*
Toluene (alone)	2	*Prunus serotina*	3	25	131.8	113.4	86.0
Do	2	do	3	25	131.8	100.0	79.5
Do	2	White Italian broomcorn	1	25	26.5	24.3	91.7
Do	4	Hegari	1	9	17.75	19.4	109.3
Glucose	4	*P. serotina*	1	37	72.4	72.4	100.0
Thymol	4	do	1	37	72.4	73.4	101.4
Hexone	8	do	1	37	72.4	72.4	100.0
Aniline	2	White Italian broomcorn	3	25	26.5	23.8	89.8
Do	4	*P. serotina*	3	25	161	161	100.0
p-Toluidine	4	do	3	25	161	149	92.5
Pyridine	4	do	3	25	161	144.8	89.9
Formaldehyde	4	Hegari	1	37	7.56	[1]	[1]
Do	1	do	1	37	7.56	1.76	23.3
Hexamine	1	do	2	9	32.1	9.7	30.2
Do	1	Spur feterita	2	9	22	8	36.4
Tyrosine	0.5	Hegari	[2]	[2]	7.56	7.02	92.9

[1] Not titratable.
[2] 24 hours at 37° followed by 17 hours at 9°.

It was thought worth while to determine whether very weak alkalies such as the organic bases—aniline, *p*-toluidine, and pyridine—might be effective in preventing the loss of hydrocyanic acid without interfering with cyanogenesis. It was found that these substances were effective as compared with inorganic alkalies and might be worthy of further investigation. All of them have the disadvantage that they distill with the hydrocyanic acid and may interfere in the titration.

Formaldehyde, hexamine, and tyrosine were employed in some tests to determine whether the addition of these substances might not increase the quantity of hydrocyanic acid by actual phytosnythesis. Except in the test with tyrosine there was a great decrease of hydrocyanic acid, and in the experiment in which 4 percent of formaldehyde was used so much of the aldehyde was present in the distillate that the reagent was immediately reduced to colloidal silver and no titration could be made. In spite of the low temperature at which the hexamine mixtures were stored there was a considerable loss of hydrocyanic acid, indicating a very direct effect of hexamine on cyanogenesis.

ALCOHOL IN VARIOUS DILUTIONS

The well-known fact that ethyl alcohol in the higher concentrations inhibits the action of enzymes raised a question whether the concentration effective for preserving cyanogenetic plants would not also inhibit the cyanogenesis to such an extent that this feature could not be overcome by subsequent handling of the samples. A series of experiments was conducted to determine (1) whether alcohol possesses sufficient preservative action to be useful, (2) in how low a concentration alcohol might still be effective, (3) how long the preservative action continues, and (4) whether these properties differ with different species of plants. The alcohol used was U. S. P. grade ethanol, which was diluted with distilled water to the desired concentration. The samples were weighed into the standard jars, covered with 300 cc of

the diluted alcohol, and set aside at 25° C. for the time indicated. Three samples of fresh arrowgrass were preserved at Salina, Utah, and shipped by express to the laboratory and so were exposed to various temperatures incidental to transport across the country during August. The data, reported in table 8, show that alcohol is a good preservative for short periods in low concentrations and for the four species represented by the plants studied. Table 9 contains a rearrangement of certain of these data to show the effect of different concentrations of alcohol with different species. In concentrations of 25 percent and lower alcohol gave good results. The higher concentrations used indicated some decrease of the cyanogenesis presumably by inhibition of enzyme action.

TABLE 8.—*Hydrocyanic acid recovered from fresh plants after storage with varying percentages of alcohol for different periods*

Alcohol (percent)	Plant	Period of preservation	Temperature of preservation	Hydrocyanic acid per 100 g of plant in—		Hydrocyanic acid recovered
				Standard	Test specimen	
		Days	*°C.*	*Milligrams*	*Milligrams*	*Percent*
10	Sudan grass	3	25	116.6	114.5	98.2
	Do	6	25	116.6	114.5	98.2
	Prunus serotina	3	25	161	168.5	104.6
15	White Italian broomcorn	4	25	35.6	33.5	94.1
	Do	3	25	26.5	24.8	93.6
	Sudan grass	3	25	116.6	116.6	100.0
	P. serotina	3	25	161	175	108.7
	Do.[1]	7	25	116.6	108.6	93.1
	Arrowgrass	6	(2)	163.8	153.4	93.6
	Hegari	14	25	19.7	21.3	108.1
	Do.[1]	21	25	10	9.2	92.0
	Spur feterita	7	25	26.5	28.6	107.9
20	Sudan grass	3	25	116.6	115.6	99.1
	Do	6	25	116.6	113.4	97.2
	Spur feterita	7	25	26.5	27.6	104.1
	Do	7	25	26.5	28	105.7
25	Sudan grass	3	25	116.6	108	92.6
	P. serotina	3	25	131.8	131.8	100.0
	Do	3	25	131.8	140.4	100.6
50	White Italian broomcorn	3	25	26.5	25.4	95.8
	Sudan grass	3	25	116.6	98.2	84.2
	Arrowgrass	6	(2)	163.8	142.8	87.2
	Do	6	(2)	163.8	144.7	88.3
95	White Italian broomcorn	3	25	26.5	14	52.8
	P. serotina	3	25	131.8	92	69.8

[1] Frosted.

[2] See footnote 1, table 1.

TABLE 9.—*Hydrocyanic acid recovered from fresh plants after storage in alcohol of varying strength for 3 to 7 days*

Alcohol (percent)	Hydrocyanic acid recovered from—				
	White Italian broomcorn	Spur feterita	Sudan grass	*Prunus serotina*	Arrowgrass
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
10			98.2 98.2	104.6	
15	94.1 93.6	107.9	100.0	108.7 [1] 93.1	93.6
20		104.1	99.1 97.2		
25		105.7	92.6	100.0	
50	95.8		84.2	100.6	87.2
95	52.8			69.8	88.3

[1] Frosted.

The question of the length of time that alcohol might preserve these plants was studied with reference to the three most suitable concentrations—15, 20, and 25 percent. Although 10 percent alcohol had given good results for short periods it was discarded after one trial because of the fact that the moisture in the plant sample, approximately 80 percent on the average, would seriously dilute 10-percent alcohol and the probability is that such a strength would not preserve against bacterial decomposition. Comparison of the effects of 15-, 20-, and 25-percent concentrations on fresh leaves of Spur feterita for 10 weeks are shown in table 10. At the end of the first week there was a small increase in the quantity of hydrocyanic acid obtained as compared with the standard, which was obtained after 24 hours of incubation of the fresh plant in water at 37° C. The rise indicates that cyanogenesis continued for several days and that the 24-hour figure did not represent the true value for this sample. By the end of the second week the yield of hydrocyanic acid began to decrease and from then on there was a steady loss of hydrocyanic acid until the seventh or eighth week, when an equilibrium appeared to have been established after a loss of approximately 20 percent of the original hydrocyanic acid. Similar comparisons were made with white Italian broomcorn and *Prunus serotina*, the results of which are reported in tables 11 and 12.

TABLE 10.—*Hydrocyanic acid recovered from fresh leaves of spur feterita after storage in alcohol of various strengths for different periods*

[Standard: 26.5 mg of hydrocyanic acid per 100 g of plant]

Period of preservation (days)	Hydrocyanic acid per 100 g of plant preserved in—			Period of preservation (days)	Hydrocyanic acid per 100 g of plant preserved in—		
	15-percent alcohol	20-percent alcohol	25-percent alcohol		15-percent alcohol	20-percent alcohol	25-percent alcohol
	Milligrams	*Milligrams*	*Milligrams*		*Milligrams*	*Milligrams*	*Milligrams*
7	28.6	27.6	28.0	42	22.7	22.7	23.3
14	26	26	27	56	21.6	21.1	21.6
21	24.8	25.4	26	70	22.2	22.2	21.1
28	24.4	24.4	24.4				

TABLE 11.—*Hydrocyanic acid recovered from white Italian broomcorn after storage in 15- and 25-percent alcohols for different periods*

[Standard: 26.5 mg of hydrocyanic acid per 100 g of plant]

Period of preservation (days)	Hydrocyanic acid per 100 g of plant preserved in—		Period of preservation (days)	Hydrocyanic acid per 100 g of plant preserved in—		Period of preservation (days)	Hydrocyanic acid per 100 g of plant preserved in—	
	15-percent alcohol	25-percent alcohol		15-percent alcohol	25-percent alcohol		15-percent alcohol	25-percent alcohol
	Milligrams	*Milligrams*		*Milligrams*	*Milligrams*		*Milligrams*	*Milligrams*
10	22.6		45	18.4		87	16.7	14
17	19.4		52	18.4		101	16.2	
24	19.4		59	18.4	17.3	115	14.3	13.2
31	20	21.2	73	17.8		143	15.9	14
38	17.8							

TABLE 12.—*Hydrocyanic acid recovered from Prunus serotina after storage in alcohol of different strengths for different periods*

[Standard: 161 mg of hydrocyanic acid per 100 g of plant]

Period of preservation (days)	Hydrocyanic acid per 100 g of plant preserved in—			Period of preservation (days)	Hydrocyanic acid per 100 g of plant preserved in—		
	10-percent alcohol	15-percent alcohol	20-percent alcohol		10-percent alcohol	15-percent alcohol	20-percent alcohol
	Milligrams	*Milligrams*	*Milligrams*		*Milligrams*	*Milligrams*	*Milligrams*
3	168.5	175	171.7	13	165.2	171.7	169.6
6	166.8	172.8	170.6	20	155.5	162	163.1

From these experiments it appeared that the use of 20- and 25-percent alcohols offered no particular advantage over 15-percent, and this strength was adopted for short-time preservation of cyanogenetic plants. A series of experiments made with fresh Sudan grass and continued for 15 weeks is reported in the following tabulation. Here there was a continuous drop for 2 weeks, when the rate of decrease diminished and equilibrium was established after 7 weeks. At that time the samples had lost approximately 60 percent of their original hydrocyanic acid.

Period of preservation (days)	Hydrocyanic acid per 100 g of plant (milligrams)	Period of preservation (days)	Hydrocyanic acid per 100 g of plant (milligrams)
0	23.1	49	8.6
7	16.2	63	8.6
14	9.8	77	8.1
21	9.8	91	8.1
28	10.2	105	7.7
35	9.2		

It should be noted here that the sample had been collected in Colorado and mailed fresh to the laboratory, where it arrived in good condition, free from mold or decomposition but undoubtedly altered in certain respects.

A sample of hegari which yielded 19.7 mg of hydrocyanic acid per 100 g after 24 hours of incubation in water was preserved in 15-percent alcohol. After 2, 11, and 19 weeks specimens yielded 21.3, 13.3, and 13.8 mg of hydrocyanic acid per 100 g, respectively, or recoveries of 108.1, 67.5, and 70.1 percent.

From the data presented it appears that although 15-percent alcohol preserves samples for 7 to 10 days without serious loss, it cannot be depended on for longer periods. However, data subsequently obtained show clearly that the figures for the hydrocyanic acid content of plants, obtained after incubation, even for as long as 24 hours, are not necessarily accurate. Data obtained by the use of 15-percent alcohol have a certain value for purposes of comparison but more than this cannot be expected of them.

MERCURIC CHLORIDE

It appeared very early in this investigation that if use could be made of the property of mercuric chloride by which it reacts with

cyanides to form nonionized mercuric cyanide, which is one of the most stable of all cyanides, loss of hydrocyanic acid in preserved samples could be prevented for long periods and a solution of the problem reached. Consideration of the use of this substance, however, brought up a number of questions. Aside from the uncertainty whether mercuric cyanide would be stable in a mixture of plant constituents, the nature and action of many of which are quite unknown, there arose the problem (1) whether mercuric chloride might not inactivate the enzymes of the plant and so prevent cyanogenesis, and (2) the quantitative recovery of hydrocyanic acid from the mixture after storage. Since mercuric cyanide is not very reactive and hydrocyanic acid is very labille, the choice of reagents and technique available to liberate the acid was narrowly limited.

Preservative Properties of Mercuric Chloride

Experiments were made to determine whether mercuric chloride actually did have any preservative properties in these plant mixtures. The general results are reported in table 13. Fresh plants were used when available, and except in two instances, 2 percent of the preservative was added in water solution and the sample was customarily diluted to 400 cc with water. It was stored at the indicated temperature for various periods and then analyzed. The standard chosen for comparison was determined as previously described and does not, of course, represent the absolute value for the hydrocyanic acid content of the specimen. With fresh plants those preserved with mercuric chloride showed a distinct increase in hydrocyanic acid recovered. Dried plants, however, after short periods of storage generally yielded smaller quantities of hydrocyanic acid, but on longer storage the quantity increased. It appeared that this effect was due to inhibition of the enzyme either by actual precipitation by the mercuric chloride or possibly by the increased hydrogen-ion concentration resulting from the hydrochloric acid liberated during the formation of mercuric cyanide. To test the latter possibility several experiments were made in which the mercuric chloride was treated with a molecular proportion of sodium citrate before addition to the plant. These buffered mixtures did not regularly increase the yield of hydrocyanic acid sufficiently to indicate that the hydrogen-ion concentration is the important factor in the inhibition. Preliminary experiments in which enzyme was added, however, showed a definite and rapid response and further study of this process was indicated. It was thought possible that some method of accelerating the enzyme action might solve the problem. Guignard (*15*) and Mirande (*16*) have reported that cyanogenetic plants treated with chloroform and other anesthetics evolve hydrocyanic acid more rapidly than untreated plants. This appeared to be due to a possible acceleration of cyanogenesis. Accordingly chloroform was added to two samples of dried Johnson grass preserved with 2-percent mercuric chloride, and the mixtures were stored for 1 and 2 says at 37° C. The results indicated a retarding effect of the chloroform. The effects reported by Guignard and Mirande therefore appear to be due to some other mechanism.

TABLE 13.—*Hydrocyanic acid recovered from plants with mercuric chloride solutions for different periods*

Mercuric chloride (percent)	Plant	Condition of plant	Period of preservation	Temperature of preservation	Hydrocyanic acid per 100 g of plant in—		Hydrocyanic acid recovered
					Standard	Test specimen	
			Days	*° C.*	*Milligrams*	*Milligrams*	*Percent*
2	Arrow grass	Dried	3	37	400.1	387	96.7
2	do	do	1	37	158.8	128	80.6
2	Hegari	Fresh	77	25	19.7	20.3	103.0
2 [1]	do	do	77	25	19.7	22	111.7
2	do	do	133	25	19.7	22	111.7
2 [1]	do	do	133	25	19.7	22.2	112.7
2	Johnson grass	Dried	13	37	294	283	96.3
			Hours				
2 [2]	do	do	4	37	294	184.7	62.8
			Days				
2 [2]	do	do	2	37	294	313	106.5
2 [3]	do	do	1	37	294	134.6	45.9
2 [3]	do	do	2	37	294	168.6	57.3
2	*Prunus melanocarpa*	do	1	37	215.3	260.8	121.1
2	do	do	62	25	178.1	196.6	110.4
2	*P. serotina*	Fresh	112	25	161	178.9	111.1
2	do	do	7	25	116.6	126.4	108.4
4	do	do	7	25	116.6	122.0	104.6
10	do	do	28	25	116.6	124.2	106.5
2	Spur feterita	Fresh	4	37	30.4	33.4	109.9
2 [2]	do	do	3	37	30.4	33.8	111.2
2 [1]	do	do	3	37	30.4	32.4	106.6
2 [1]	do	do	4	37	30.4	32.8	107.9

[1] Treated with a molecular proportion of sodium citrate before addition to the plant.
[2] Enzyme added.
[3] 2 cc of chloroform added.

INHIBITION OF CYANOGENESIS BY MERCURIC CHLORIDE

A study of the comparative preservative efficiency of 15-percent alcohol and 2- and 4-percent mercuric chloride solutions furnished evidence of the inhibitory effect of the salt. Samples of a single collection of fresh *Prunus serotina* collected near the laboratory and passed through a food chopper were preserved in the three solutions and analyzed at intervals for 22 weeks. The results are reported in table 14 and plotted in figure 1.

TABLE 14.—*Hydrocyanic acid recovered from fresh Prunus serotina leaves after storage in 15 percent alcohol and 2- and 4-percent mercuric chloride solutions for 1 to 22 weeks*

Period of preservation (weeks)	Hydrocyanic acid per 100 g of plant in—			Period of perservation (weeks)	Hydrocyanic acid per 100 g of plant in—		
	15-percent alcohol	2-percent mercuric chloride	4-percent murcuric chloride		15-percent alcohol	2-percent mercuric chloride	4-percent mercuric chloride
	Milligrams	*Milligrams*	*Milligrams*		*Milligrams*	*Milligrams*	*Milligrams*
1	108.6	126.4	122	8	----	126.5	----
2	106.9	124.2	119.9	10	94.4	127.7	123.9
4	----	125.2	----	15	----	132.1	128.4
6	100.4	126.4	121.5	22	88.7	133.2	----

The curves clearly show the excellence of the preservative action of the mercuric chloride as compared with 15-percent alcohol, and the inhibiting effect is indicated by the lower values obtained with 4-percent as compared with 2-percent mercuric chloride. The fact that in both cases there is a steady increase in hydrocyanic acid over the whole period after the first week indicates that the action of the bichloride is an inhibition rather than a destruction of the enzyme.

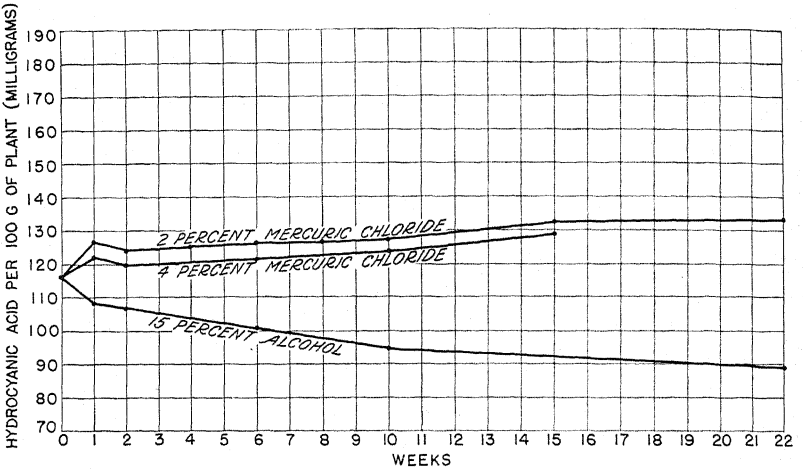


FIGURE 1.—Hydrocyanic acid recovered from fresh cherry leaves (*Prunus serotina*) preserved in 15-percent alcohol and 2- and 4-percent mercuric chloride solution.

A similar effect was observed in a series of experiments in which dried Johnson grass was used. Samples weighing 25 g were mixed with water and with a 2-percent mercuric chloride solution, and to a few samples of each enzyme was added. All were stored at 37° C. and analyzed at intervals. The samples that were mixed with water only were withdrawn at 2-hour intervals during the first day and analyzed for hydrocyanic acid content; the rest of these samples and samples that were mixed with mercuric chloride solution were withdrawn at longer intervals. The results are reported in table 15. Here the inhibitory effect of the bichloride is evident. When the plant was incubated with water alone the highest yield of hydrocyanic acid was obtained on the sixth day, whereas in the case of the bichloride the yield of hydrocyanic acid after 6 days was only 90.7 percent of that in water and the comparable yield was not obtained until the thirteenth day. At the end of 24 hours, however, samples to which enzyme had been added yielded larger quantities of hydrocyanic acid than either of the other series, and at the end of 48 hours the sample preserved in mercuric chloride to which enzyme had been added yielded the largest quantity of hycrocyanic acid obtained in the entire experiment.

The data for the samples mixed with water alone disclose a phenomenon, observed regularly in studies of this kind, which consists in a decrease in quantity of hydrocyanic beginning after some 10 hours of incubation and continuing for a variable period of 6 to 12 hours, when

the yield again reaches the former level and continues to increase. This drop has been consistently noted with several cyanogenetic plants, and it is planned to make it the subject of investigation to determine the factors which are operating.

TABLE 15.—*Rate of formation of hydrocyanic acid in dried Johnson grass stored in water and in 2-percent mercuric chloride solution at 37° C. for different periods*

Period of preservation	Hydrocyanic acid per 100 g of plant preserved in—				Period of preservation	Hydrocyanic acid per 100 g of plant preserved in—			
	Water	Mercuric chloride	Mercuric chloride and enzyme	Water and enzyme		Water	Mercuric chloride	Mercuric chloride and enzyme	Water and enzyme
Hours	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Hours*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
0	123.1				42	214.9			
2	136.6				*Days*				
4	139.8		143.6	186	2	222.4	189.7	313	
6	150.5	101.3			3	262	211.7		
8	156.6				4	267.5	232.3		
10	196.1				5	273.8	258.1		
12	181.4				6	283.7	257.4		
14	185.4				7	278.6	265.9		
16	184				8		267		
18	186.4	136.1			9		271.6		
20	188.4				10		272.9		
22	198.3				11		278.2		
24	205.1	154	294.1	294.4	12		278.9		
30	210.4				13		283		
36	220.3	169.6							

The inhibiting effect of mercuric chloride is also shown in experiments with dried arrowgrass in which results obtained with storage of plants in water are compared with results from storage in 2-percent mercuric chloride. The data are reported in table 16. They show a retardation in the formation of hydrocyanic acid at periods between the sixth and eighteenth hours. The samples preserved in mercuric chloride did not reach quite as high a yield as those preserved with water alone at the end of the experiment, when the material was exhausted.

TABLE 16.—*Rate of formation of hydrocyanic acid in dried arrowgrass stored in water and 2-percent mercuric chloride solution at 37° C. for different periods*

Period of preservation (hours)	Hydrocyanic acid per 100 g of plant preserved in—		Period of preservation (hours)	Hydrocyanic acid per 100 g of plant preserved in—	
	Water	Mercuric chloride		Water	Mercuric chloride
	Milligrams	*Milligrams*		*Milligrams*	*Milligrams*
0	149.7		16	381.9	
2	376.7		18	393.2	
4	382.1		20	400.1	
6	385.2		22	399	
10	360.5		24	394.4	381.8
12	391	378.3	48	402.4	387.9
14	391		72		387

Since it was possible to obtain 313 mg of hydrocyanic acid per 100 g of Johnson grass by adding enzyme, whereas the use of water alone gave 283.7 mg as the highest, it is evident that either cyanogenesis was incomplete in the water solution or hydrocyanic acid was lost in some manner. If the latter be true it follows that the figures obtained in the analysis of cyanogenetic plant mixtures with water represent the resultant of two processes: (1) the formation of hydrocyanic acid by cyanogenesis, and (2) the disappearance of hydrocyanic acid either by hydrolysis to ammonium formate or conversion into some other compound. The disappearance of hydrocyanic acid under circumstances in which loss by volatilization is excluded, as in closed systems, was illustrated in a series of determinations made on dried Sumac sorghum, in which weighed samples of plant were mixed with water, stored at 37° C., and withdrawn at 2-hour intervals for 24 hours, omitting the 8-hour determination. The samples were analyzed for hydrocyanic acid content immediately upon withdrawal. The results are reported in the following tabulation:

Period of storage (hours)	Hydrocyanic acid per 100 g of plant (milligrams)	Period of storage (hours)	Hydrocyanic acid per 100 g of plant (milligrams)
0	83.2	14	74.5
2	78.8	16	71.3
4	77.8	18	67.4
6	77.8	20	65.8
10	73.4	22	59.4
12	75.6	24	34.6

The loss of 58.4 percent may be considered to represent only a part of the actual loss of hydrocyanic acid in this case since it is not likely that the first figure, 83.2 mg, includes the total potential hydrocyanic acid present in the plant. The fact that such large losses can occur was confirmed with other specimens of dried sorghums.

That this loss is not always apparent and that data obtained in similar experiments may be misleading were indicated in a series of experiments conducted on a sample of dried (*Prunus melanocarpa*, in which 25-g samples were stored with water and with 2-percent mercuric chloride solutions and analyzed at intervals for 24 hours. The results are reported in table 17 and are plotted in figure 2. The curve for hydrocyanic acid in the samples stored in water over the 24-hour period again exhibits a depression similar to that noted in the case of Johnson grass with a recovery and subsequent drop. The loss of hydrocyanic acid after 24 hours as shown by the data is only 5 percent, much less than was noted with Sumac sorghum. However, the curve for the portion of the sample preserved in mercuric chloride shows that the true value for the hydrocyanic acid content of the sample was at least 260.5 mg. There was, then, a loss of at least 45.2 mg, or 17.4 percent, during the 24-hour period. Such considerations raise the serious question whether any hydrocyanic acid determination is reliable in which account of the possible loss of hydrocyanic acid during analysis has not been taken.

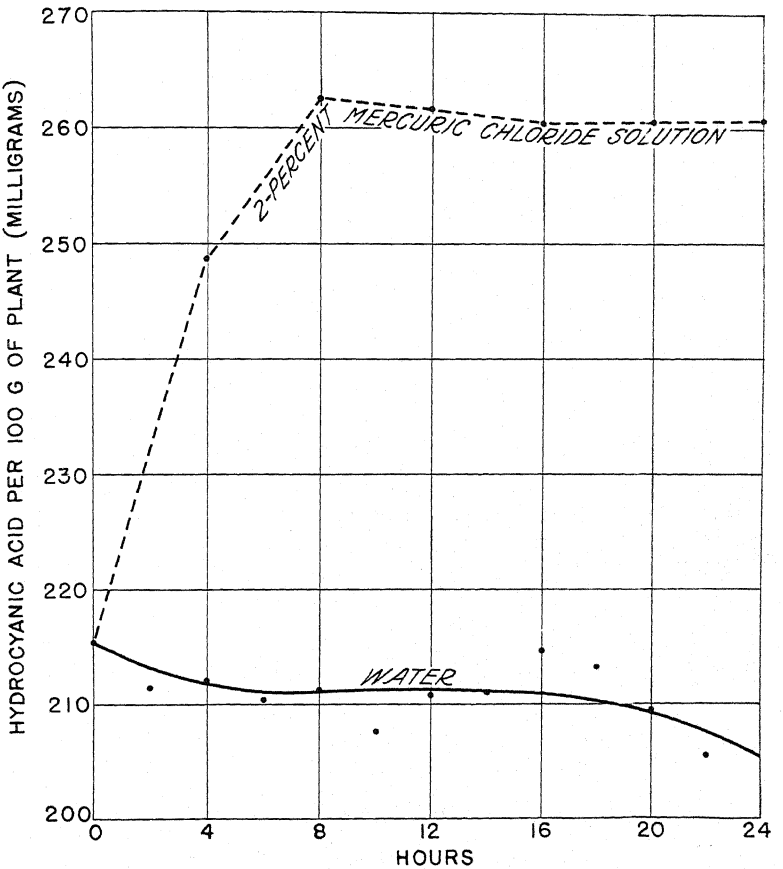


FIGURE 2.—Rate of formation of hydrocyanic acide from dried cherry leaves (*Prunus melanocarpa*) stored in water and in 2-percent mercuric chloride solution.

TABLE 17.—*Rate of formation of hydrocyanic acid in dried Prunus melanocarpa stored in water and in 2-percent mercuric chloride solution for different periods*

Period of storage (hours)	Hydrocyanic acid per 100 g of plant preserved in—		Period of storage (hours)	Hydrocyanic acid per 100 g of plant preserved in—		Period of storage (hours)	Hydrocyanic acid per 100 g of plant preserved in—	
	Water	Mercuric chloride		Water	Mercuric chloride		Water	Mercuric chloride
	Milligrams	*Milligrams*		*Milligrams*	*Milligrams*		*Milligrams*	*Milligrams*
0	215.3		10	207.8		18	213.2	
2	211.6		12	210.9	261.6	20	209.5	260.7
4	212.1	248.6	14	211		22	205.5	
6	210.5		16	214.6	260.5	24	205.1	266.8
8	211.1	262.6						

PERIOD OF EFFECTIVE PRESERVATION BY MERCURIC CHLORIDE

A number of experiments were made to determine how long the hydrocyanic acid content of plants treated with mercuric chloride

solutions may be preserved. Samples of fresh plants were collected, minced in the usual way, thoroughly mixed, and weighed into preservative jars, and immediately mixed with 1 percent of mercuric chloride in solution. At the same time, as controls, untreated samples were selected for determination of hydrocyanic acid. The preserved samples were stored at 25° C. and specimens were taken for analysis after various periods. Data for samples of *Prunus serotina* leaves and of four varieties of sorghum are reported. The standard was determined by macerating the ground sample for 24 hours in water at 37° and then distilling. The results of representative experiments are given in table 18. The figures show good preservation over a period of 6 months. In one instance the quantity of hydrocyanic acid recovered from a preserved sample was less than for the previous period of storage, but this result may have been due to an error of sampling. The uniformly higher figures obtained after 4 weeks of preservation as compared with the standard again emphasize the error in determinations made by the customary technique.

TABLE 18.—*Hydrocyanic acid recovered from Prunus serotina and sorghum varieties after storage in 1-percent solutions of mercuric chloride for different periods*

Plant	Hydrocyanic acid in 100 g of fresh plant in—				Plant	Hydrocyanic acid in 100 g of fresh plant in—			
	Standard	Test specimens stored for—				Standard	Test specimens stored for—		
		4 weeks	12 weeks	6 months			4 weeks	12 weeks	6 months
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*		*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
Chiltex	14.6	16.3	-------	16.4	Spur feterita	10.4	11.6	12.5	12.7
Kansas Orange	15.3	15.7	-------	15.8		26.3	32.3	31.3	33.3
Prunus serotina	98.6	111.7	114.0	-------	Sumac	8.0	12.0	12.0	-------
	112.1	121.0	-------	-------					

STRENGTH OF MERCURIC CHLORIDE NECESSARY FOR PRESERVATION

The strength of mercuric chloride needed to insure preservation was the subject of several series of experiments with dried and fresh sorghums. Since preservation was found to exert a retarding effect on cyanogenesis, it is desirable to reduce the strength as much as possible without loss of preservative power. In the experiments different quantities of mercuric chloride were used and the storage periods were extended as long as the specimen material lasted. The results, reported in table 19, indicate that 1 percent, calculated on the weight of plant, is necessary for fresh plants. In the case of dried plants, 2 percent preserved reasonably well for 3 months, but after that time there was a considerable loss. indicating hat a higher percentage is required for dried sorghum. For dried cherry (*Prunus melanocarpa*) 2-percent mercuric chloride preserved satisfactorily for 6 months (table 20).

TEMPERATURE OF STORAGE IN MERCURIC CHLORIDE

Experiments conducted on dried samples of *Prunus melanocarpa* stored in mercuric chloride at 9°, 25°, and 37° C. indicated a small

advantage for those stored at 25°. These results are reported in table 20. A series of samples stored with water at 37° for comparison shows the great loss of hydrocyanic acid that occurs under these conditions.

TABLE 19.—*Hydrocyanic acid recovered from sorghums preserved with mercuric chloride at 37° C. in varying concentrations for different periods*

Plant	Period of preservation	Hydrocyanic acid per 100 g of plant preserved in—					
		Water	Mercuric chloride				
			0.25 percent	0.5 percent	0.75 percent	1 percent	2 percent
		Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
Fresh hegari	0	11.7					
Do	24 hours	11.4					
Do	2 weeks		15.8	15.5	15.6	15.8	
Do	3 weeks		17	18.1	19.4	20.8	
Do	5 weeks		15.5	20.2	18.6	20.4	
Do	7 weeks		13	[1] 24.2	18.4	25.9	
Do	9 weeks			19.4	19.8	22.5	
Dried hegari	0	32					
Do	24 hours	31.8					
Do	4 weeks			54.8		61.8	69.7
Do	3 months			44.4		47.9	67.6
Do	5 months			30.6		34.9	55.3
Do[2]	0	30.7					
Do	24 hours	37.1					
Do	1 week			63.8		70.2	69.4
Do	2 weeks			61.2		66.8	69.9
Do	3 weeks			55.6		67.3	70.1
Do	4 weeks			54.3		64.2	70.2
Dried Chiltex	0	51.3					
Do	24 hours	66.2					
Do	4 weeks						82.6
Do	3 months						81.2
Do	5 months						56.3
Do[3]	0	54.1					
Do	24 hours	73.4					
Do	1 week						91.75
Do	2 weeks						92.7
Do	3 weeks						93.2
Do	4 weeks						95

[1] End point obscured by darkening.
[2] Whole sample, finely ground.
[3] Finely ground; coarse fibers discarded.

TABLE 20.—*Hydrocyanic acid recovered from dried Prunus melanocarpa after storage in 2-percent mercuric chloride solution at various temperatures for different periods*

Period of preservation	Hydrocyanic acid per 100 g of plant preserved in—				Period of preservation	Hydrocyanic acid per 100 g of plant preserved in—			
	Water at 37° C.	2-percent mercuric chloride at—				Water at 37°C.	2-percent mercuric chloride at—		
		9° C.	25° C.	37° C.			9° C.	25° C.	37° C.
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*		*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
0	162.5				4 months	84.9	196.4	201.8	196.3
24 hours	178.1				6 months	71.5	199	201.4	190.2
2 months		194.4	197.6	196.6	8 months	61.7		197.2	

RECOVERY OF HYDROCYANIC ACID FROM MERCURIC CYANIDE

One of the most important problems connected with the mercuric chloride method of preservation was the quantitative recovery of hydrocyanic acid from the preserved samples. Two procedures have been reported and both were studied in the present investigation. Rupp and Goy (*22*) suggested the use of potassium iodide to convert mercuric cyanide into mercuric iodide and hydrocyanic acid, and Roe (*19*) advocated the use of stannous chloride, which converts mercuric cyanide into calomel and hydrocyanic acid. Both reactions take place in slightly acid solution, the normal condition of these plant mixtures. Because of its stability and ease of application, potassium iodide was first used to free the hydrocyanic acid for analysis in this study. The results appeared to be satisfactory for the greater number of plants, but a serious difficulty arose with fresh sorghums. With these samples a cloudy precipitate formed in the second and later fractions of the distillate, making titration impossible, and solid matter of a greenish-black color accumulated in the coils of the Friedrichs condensers used. It was impracticable to filter the precipitate from the distillates so that, although the greater portion of the hydrocyanic acid might be distilled into the first distillate and be determined without difficulty, it was not possible to estimate accurately the small fraction that distilled later.

Study of the interfering substances showed that the precipitate in the distillate consisted of mercuric iodide, and the deposit in the condensers proved to be a mixture of mercurous iodide and metallic mercury. The authors were unable to find a recorded observation of the volatility of mercurous iodide with steam. Distillation of a mixture of potassium iodide and calomel in water led to the formation of a similar deposit in the condenser and of mercuric iodide in the distillate. It was concluded that mercurous iodide, distilling into the condenser, reacts with water to form mercury and mercuric iodide. The latter is washed into the receiver; the former remains with the excess of mercurous compound and may be seen with the aid of a lens in characteristic globules. The mercurous iodide may be dissolved out with potassium iodide solution.

Stannous chloride did not possess this disadvantage, although a minute quantity of mercury compound, presumably calomel, is carried over with the vapors and collects in the condenser as a black film visible after several distillations but in so small a quantity that it does not interfere with the accuracy of the determination or affect the titration in any manner. A series of parallel determinations, potassium iodide being used in one set and stannous chloride in the other, revealed the superiority of the latter as a reagent for the decomposition of mercuric cyanide. The data are reported in table 21, which shows the greater efficiency of stannous chloride except in the case of dried Johnson grass, in which that reagent was distinctly less efficient than potassium iodide. The reason for this discrepancy is not clear.

TABLE 21.—*Recovery of hydrocyanic acid from mercuric cyanide using potassium iodide and stannous chloride*

Plant	Quantity of hydrocyanic acid per 100 g of plant liberated by the use of—		Relative efficiency of stannous chloride to potassium iodide	Plant	Quantity of hydrocyanic acid per 100 g of plant liberated by the use of—		Relative efficiency of stannous chloride to potassium iodide
	Potassium iodide [1]	Stannous chloride [2]			Potassium iodide [1]	Stannous chloride [2]	
	Milligrams	*Milligrams*			*Milligrams*	*Milligrams*	
Hegari	[3] 15	[3] 22	1.47	Feterita	23.8	29.2	1.23
Do	13.6	20.3	1.49	*Prunus serotina*	132.1	133.9	1.01
Johnson grass	100.6	69.3	.69	*Prunus melanocarpa*	186.0	200.7	1.08
Do	[4] 106.7	-------	-------	Arrowgrass	102.8	132.0	1.28
Do	135.5	100	.73				

[1] 4 moles. [2] 2.1 moles. [3] Buffered. [4] 6 moles.

Recovery of hydrocyanic acid from potassium cyanide after treatment of the latter with mercuric chloride was studied with stannous chloride and potassium iodide as reagents. In one case ammonium chloride was added to the stannous chloride in proportion to form the double salt $SnCl_2 . 2 NH_4Cl$, in an effort to stabilize the tin compound. However, it was found that no advantage in stability was gained, although there was a slight advantage in recovery of hydrocyanic acid. The results are reported in table 22. With potassium iodide the efficiency of recovery was lower than with stannous chloride, and here again mercurous iodide distilled into the condenser and rendered the distillate turbid.

The loss of hydrocyanic acid during these operations suggested that the substance might be decomposed in the distilling flask by hydrochloric acid. A series of experiments was conducted to determine the effect of small quantities of vraious acids on known quantities of hydrocyanic acid under conditions similar to those obtaining in the analytical procedure. The acids, in solution, were added to cold solutions of potassium cyanide. In some cases the mixtures were immediately distilled. In others they were refluxed for from 1 to 2 hours in closed systems, and then the hydrocyanic acid formed was distilled into an alkaline solution and titrated. The results are reported in table 23.

These results indicate that in the ordinary course of analysis some of the hydrocyanic acid is decomposed during the process of distillation and that the value obtained will be low. Bishop (*6*) reports losses ranging from 3 to 5 percent on distilling mixtures of potassium cyanide and dilute acids. Apparently the presence of mercuric chloride or its reaction products and of stannous chloride in the system does not seriously increase the loss from this source.

TABLE 22.—*Recovery of hydrocyanic acid from mercuric cyanide by the use of various reagents*

Reagent	Hydrocyanic acid			Reagent	Hydrocyanic acid		
	Present	Recovered			Present	Recovered	
	Milligrams	*Milligrams*	*Percent*		*Milligrams*	*Milligrams*	*Percent*
Stannous ammonium chloride ($SnCl_2.2NH_4Cl$)	232.1	229.45	98.9	Stannous chloride (excess)	240.5	235.2	97.8
Stannous chloride (1 mole)	239.1	233.6	97 7	Potassium iodide (excess) [1]	240.5	228.1	94.8
Stannous chloride (2 moles)	239.1	234.7	98.2				

[1] Distillate turbid.

TABLE 23.—*Recovery of hydrocyanic acid after heating with 0.5 percent of different acids*

Acid	Time of distillation	Hydrocyanic acid		
		Present	Recovered	Lost
	Minutes	*Milligrams*	*Milligrams*	*Percent*
Oxalic	45	59.24	57.84	2.37
Oxalic (refluxed 1 hour)	45	59.35	40.88	31.13
Phosphoric	45	59.24	55.55	6.23
Sulphuric	40	59.30	55.65	6.15
Sulphuric (refluxed 1.5 hours)	45	59.19	41.58	29.76
Tartaric (refluxed 2 hours)	45	59.33	40.88	31.10
Do	40	59.31	55.44	6.52

SUMMARY AND CONCLUSIONS

Fresh cyanogenetic plants stored at ordinary temperatures without preservatives lost 13 to 83 percent of their hydrocyanic acid in 1 to 6 days.

When stored at refrigerator temperatures for 1 to 5 days, fresh spur feterita yielded as much hydrocyanic acid as before storage, but from one-third to one-half less than was obtained by 24-hour maceration of nonrefrigerated plants. Maceration of refrigerated plants leads to great loss of hydrocyanic acid.

When fresh plants were stored in water with the addition of chloroform, with or without added alcoholic potassium hydroxide or water, losses up to 67 percent occurred in all but two samples. With hegari chloroform was effective as a preservative for 4 days in one sample.

Tests with acid solutions usually resulted in rapid loss of hydrocyanic acid. Salicylic acid preserved most effectively of the acids tested.

Tests with alkaline solutions resulted in losses of from 32 to 96 percent. Organic bases, such as aniline, *p*-toluidine, and pyridine, were more effective. The losses noted in alkaline solut ons appear to be due to destruction of hydrocyanic acid rather than to inhibition of cyanogenesis.

Alcohol in concentrations of 10, 15, 20, and 25 percent preserved for 3 to 7 days with losses up to 8 percent. After a week the losses were larger until, after 7 to 8 weeks, 20 percent of the hydrocyanic acid had been lost. Alcoholic preservation cannot be relied on to

develop the maximum yield of hydrocyanic acid potential in the plant. Alcohol in concentrations of 50 and 95 percent inhibited cyanogenesis.

Mercuric chloride in water solution proved to be an excellent preservative when used in the proportion of 1 percent by weight for fresh plant. Specimens so preserved and stored for 6 months have shown no loss of hydrocyanic acid. Lower concentrations of mercuric chloride either did not preserve or led to variable results. A concentration of 2-percent mercuric chloride preserved dried plants reasonably well for 3 months. With dried sorghums and for longer periods, a higher, undetermined concentration would be required.

Plants preserved with mercuric chloride generally yielded more hydrocyanic acid than when not so preserved except in certain cases in which the experiments may have been terminated before the maximum yield of hydrocyanic acid was obtained.

In the concentrations used mercuric chloride retarded but did not stop enzyme action in cyanogenetic plants. Buffering did not counteract this effect and the addition of chloroform did not accelerate enzymolysis. The addition of enzyme greatly accelerated the rate of hydrocyanic acid formation in the presence of mercuric chloride.

The rate of formation of hydrocyanic acid in cyanogenetic plants is not uniform. A drop in hydrocyanic acid yield begins about 8 to 10 hours and continues for several hours before the yield again reaches the level observed after the first 8 hours. The period that elapses between the beginning and recovery lasts for 10 to 12 hours. This phenomenon is very characteristic and has been noted in all the plants studied.

The figure for the hydrocyanic acid content of plants determined after maceration with water or diluted alcohol is the resultant of two processes—cyanogenesis and conversion of freed hydrocyanic acid into other compounds, often referred to as destruction of hydrocyanic acid. The value obtained may be considerably below the true figure.

Large quantities of hydrocyanic acid are converted into other compounds when hydrocyanic acid is heated with very dilute acids. This results in an unavoidable error in the determination of hydrocyanic acid in plants.

The optimum temperature for storage of samples preserved with mercuric chloride solution, as between a refrigerator at 9° C., the laboratory at 25°, and an incubator at 37°, was found to be 25°.

An analytical technique for recovering hydrocyanic acid from samples preserved with mercuric chloride was developed. To liberate hydrocyanic acid from mercuric cyanide, stannuous chloride was found to be preferable to potassium iodide except in one instance.

Mercurous iodide appears to be volatile with steam and deposits in condensers where it reacts with water to form mercuric iodide and metallic mercury. The mercuric iodide may render distillates turbid and interfere with the end point in titration.

LITERATURE CITED

(1) ACHARYA, C. NARASIMHA.
1933. INVESTIGATIONS ON THE DEVELOPMENT OF PRUSSIC ACID IN CHOLAM (SORGHUM VULGARE). Indian Jour. Agr. Sci. 3: 851–869, illus.

(2) ALSBERG, CARL L., and BLACK, OTIS F.
1915. CONCERNING THE DISTRIBUTION OF CYANOGEN IN GRASSES, PARTICULARLY IN THE GENERA PANICULARIA OR GLYCERIA AND TRIDENS OR SIEGLINGIA. Jour. Biol. Chem. 21: 601–609.

(3) ——— and BLACK, OTIS F.
1916. THE SEPARATION OF AUTOGENOUS AND ADDED HYDROCYANIC ACID FROM CERTAIN PLANT TISSUES AND ITS DISAPPEARANCE DURING MACERATION. Jour. Biol. Chem. 25: 133–140.

(4) AULD, S. J. MANSON.
1908. THE HYDROLYSIS OF AMYGDALIN BY EMULSIN, PART I. Jour. Chem Soc. [London] Trans. 93: 1251–1275, illus.

(5) AULD, S. J. M.
1913. CYANOGENESIS UNDER DIGESTIVE CONDITIONS. Jour. Agri. Sci. [England] 5: [409]–417.

(6) BISHOP, L. R.
1927. THE ESTIMATION OF CYANOGENETIC GLUCOSIDES. Biochem. Jour. 21: 1162–1167.

(7) BRÜNNICH, J. C.
1903. HYDROCYANIC ACID IN FODDER-PLANTS. Jour. Chem. Soc. [London] Trans. 83: 788–796, illus.

(8) CALDWELL, ROBERT J., and COURTAULD, STEPHEN LEWIS.
1907. THE HYDROLYSIS OF AMYGDALIN BY ACIDS. Jour. Chem. Soc. [London] Trans. 91: 666–671.

(9) CHARLTON, J.
1926. THE SELECTION OF BURMA BEANS (PHASEOLUS LUNATUS) FOR LOW PRUSSIC ACID CONTENT. India Dept. Agr. Mem., Chem. Ser. 9: 1–36.

(10) CLAWSON, B. J., and YOUNG, C. C.
1913. PRELIMINARY REPORT ON THE PRODUCTION OF HYDROCYANIC ACID BY BACTERIA. Jour. Biol. Chem. 15: 419–422.

(11) COLLINS, S. H.
1912. THE RATE OF EVOLUTION OF HYDROCYANIC ACID FROM LINSEED UNDER DIGESTIVE CONDITIONS. Univ. Durham Phil. Soc. Proc. 4: 99–106.

(12) DENIGÈS, G.
1893. MÉTHOD GÉNÉRAL POUR LA DOSAGE VOLUMÉTRIQUE DE L'ARGENT SOUS UNE FORME QUELCONQUE. Compt. rend. Acad. Sci. [Paris] 117: 1078–1081.

(13) DEZANI, S.
1911. LA RIDUZIONE DELL'ACIDO CIANIDRICO AD OPERA DEI SUCCHI DELLE PIANTE. Atti. Cong. Naz. Chim. Appl. Turin, pp. 368–378.

(14) EMERSON, H. W., CADY, H. P., and BAILEY, E. H. S.
1913. ON THE FORMATION OF HYDROCYANIC ACID FROM PROTEINS. Jour. Biol. Chem. 15: 415–417.

(15) GUIGNARD, L.
1909. INFLUENCE DE L'ANESTHESIE ET DU GEL SUR LE DÉDOUBLEMENT DE CERTAINES GLUCOSIDES CHEZ LES PLANTES. Compt. Rend. Acad. Sci. [Paris] 149:91–93.

(16) MIRANDE, MARCEL.
1909. INFLUENCE EXERCÉE PAR CERTAINES VAPEURS SUR LA CYANOGENÈSE VÉGÉTALE. PROCÉDÉ RAPIDE POUR LA RECHERCHE DES PLANTES À ACIDE CYANHYDRIQUE. Compt. Rend. Acad. Sci. [Paris] 149:140–142.

(17) RAVENNA, C., and BABINI, V.
1912. SULLA PRESENZA DELL'ACIDO CIANIDRICO LIBERO NELLA PIANTI. Atti R. Accad. Lincei, Rend., Cl. Sci. Fis., Mat, e Nat. (5) 21:540–544.

(18) ——— and TONEGUTTI, M.
1910. ALCUNE OSSERVAZIONI SULLA PRESENA DELL'ACIDO CIANIDRICO LIBERO NELLA PIANTE. Atti R. Accad. Lincei, Rend., Cl. Sci. Fis., Mat. e Nat. (5) 19:19–25.

(19) ROE, JOSEPH H.
1924. THE ESTIMATION OF THE HYDROGEN CYANIDE CONTENT OF AMYGDALIN BY THE AERATION METHOD. Jour. Biol. Chem. 58:667–669.

(20) ROSENTHALER, L.
1919. BEITRÄGE ZUR BLAUSÄURE-FRAGE. 1. UMWANDLUNG DER BLAUSÄURE DURCH PFLANZENSÄFTE. Schweiz. Apoth. Ztg. 57:[267]–270.

(21) ———
1919. BEITRÄGE ZUR BLAUSÄUREFRAGE. 3. IN WELCHER FORM KOMMT BLAUSAURE IN PFLANZENREICH VOR? Schweiz Apoth. Ztg. 57:[571]–576.

(22) RUPP, E., and GOY, S.
1909. UEBER DAS QUECKSILBEROXYCYANID. II MITTEILUNG: UMSETZUNG MIT HALOGEN UND HALOGENVERBINDUNGEN. Arch. Pharm. 247:100–107.

(23) SWANSON, C. O.
1921. HYDROCYANIC ACID IN SUDAN GRASS. Jour. Agr. Research 22: 125–138.

(24) TAMMANN, G.
1892. DIE REACTIONEN DER UNGEFORMTEN FERMENTE. Hoppe-Seyler's Ztschr. Physiol. Chem. 16:[271]–328.

(25) TREUB, M.
1896. SUR LA LOCALISATION, LE TRANSPORT ET LE RÔLE DE L'ACIDE CYANHYDRIQUE DANS LE PANGIUM EDULE REINW. Ann. Jard. Bot. Buitenzorg 13:1–89, illus.

(26) VIEHOEVER, ARNO, JOHNS, CARL O., and ALSBERG, CARL L.
1916. CYANOGENESIS IN PLANTS. STUDIES ON TRIDENS FLAVUS (TALL RED TOP). Jour. Biol. Chem. 25:141–150.

(27) WALKER, J. WALLACE and KRIEBLE, VERNON K.
1909. THE HYDROLYSIS OF AMYGDALIN BY ACIDS. PART 1. Jour. Chem. Soc. [London] Trans. 95:1369–1377.

(28) WARTH, F. J.
1923. A NOTE ON HYDROCYANIC ACID IN THE BURMA BEAN (PHASEOLUS LUNATUS SP.). India Dept. Agr. Mem., Chem. Ser. 7:1–29, illus.

(29) WILLAMAN, J. J.
1917. THE ESTIMATION OF HYDROCYANIC ACID AND THE PROBABLE FORM IN WHICH IT OCCURS IN SORGHUM VULGARE. Jour. Biol. Chem. 29:25–36.

(30) ——— and WEST, R. M.
1916. EFFECT OF CLIMATIC FACTORS ON THE HYDROCYANIC-ACID CONTENT OF SORGHUM. Jour. Agr. Research 6:261–272, illus.

(31) WILLSTÄTTER, RICHARD, and CSÁNYI, WILHELM.
1921. ZUR KENNTNISS DES EMULSINS. Hoppe-Seyler's Ztschr. Physiol. Chem. 117:[172]–200, illus.

RELATION OF ATMOSPHERIC CONDITIONS TO ENLARGEMENT RATE AND PERIODICITY OF WINESAP APPLES [1]

By C. P. HARLEY, *physiologist*, and M. P. MASURE, *junior physiologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Since the early demonstrations of periodicity in plant growth by Sachs (*20*),[2] in 1874, many investigators have studied this phenomenon in various species of plants but comparatively few have directed their attention to tree fruits and vegetables. It was observed by MacMillan (*18*) that the enlargement of the potato tuber over a 24-hour period was not continuous but rhythmic and that the growth-rate maxima were followed by periods of slower growth or by complete cessation of enlargement. MacMillan believed that this periodic growth of the tuber was influenced by the periodic growth of the aerial portions of the plant. D. F. Fisher,[3] in 1920, obtained continuous measurements of the diameter increase of apples over 24-hour periods, and from his studies concluded that during the summer the enlargement of apples takes place mainly at night.

In recent years the rate of fruit enlargement has been used as an index of tree response to various factors affecting tree growth and behavior. Furr and Magness (*12*), working with apples, Aldrich and Work (*1*) with pears, Hendrickson and Veihmeyer (*16*) and Cullinan and Weinberger (*9*) with peaches, and many others have studied response to soil-moisture variations by measuring the circumference of the fruits at frequent intervals during the growing season. Aldrich and Work (*1*) also included in their study the influence of evaporating power of the air on growth of pears by noting the difference in circumference growth of fruit during periods of high and low evaporating power.

The value of such fruit measurements as an aid in interpreting environmental influences in the field is quite evident, and they probably will be employed to an even greater extent in the future than they have been in the past. In these researches some consideration has been given to the periodicity of fruit enlargement by taking fruit measurements at a specified time of day. Any error resulting from periodic fluctuations in rate of growth would probably be small if the intervals between measurements were 2 days or more. However, it is quite desirable in some studies to take daily measurements, in which case periodicity might become quite important.

The present study was undertaken to determine the daily enlargement rate and periodicity in growth of Winesap apples in the Wenatchee Valley, Wash., and the effect, if any, of atmospheric influences. One objective of the experiment has been the possible application of these findings to other fruit-growth studies now contemplated.

[1] Received for publication October 1, 1937; issued August 1938.
[2] Italic numbers in parentheses refer to Literature Cited, p. 123.
[3] Unpublished data in possession of writers, 1920.

METHODS AND MATERIALS

To estimate the rate of fruit growth during relatively short intervals, an apparatus was desired which would give a continuous record and which would be sufficiently sensitive to measure small changes in enlargement. An instrument was finally devised which fulfilled both of these requirements. Two of these instruments were used in the orchard to record fruit growth during the last 3 months of the 1936 growing season at Wenatchee, Wash. The apparatus was set up by encircling an apple at its greatest circumference with a strip of brass tape 0.051 mm thick, 5 mm wide, and 30 cm long. One end of this tape was widened and slotted to permit the other end to pass through. The narrow end was held in a fixed position and the broad slotted end attached to a modified hygrograph assembly. A standard hygrothermograph clock with weekly charts was used to register the movement of the pen arm. Expansion of the apple caused the tape to pull against the magnification levers of the pen arm, and the enlargement was thus recorded. A piece of 1¼-inch angle iron 20 inches long, bolted to the bottom of the case, afforded a base for clamps and also served to increase the rigidity of the assembly. The apple under observation was held by a large light-spring clamp, the jaws of which were cushioned with soft rubber tubing. It was found necessary to hold the apple rather firmly but without restricting growth.

The instruments were placed under the tree on a table that was set securely in place and staked to the ground. The main branch bearing the apples was solidly braced, and the smaller limbs were carefully held in position with clamps padded with cotton. It was essential, of course, to minimize movements of the branches by wind or other agencies in order to prevent transmission of such disturbances to the recording pen. No difficulty was experienced from this source in the present study. The apparatus as it appeared in the orchard is shown in figure 1.

Certain inherent errors, obviously present in studies of this character where physical methods are employed, may in some cases seriously limit the field of adaptability. In the present study, for example, it seemed necessary to determine and take into consideration the variations that might arise from the effect of temperature changes on the recording mechanism. The thin metal tape encircling the apple was found to have a coefficient of thermal expansion of 20×10^{-6}. The instruments were standardized both in the laboratory and in the field by substituting a porcelain sphere for the apple and noting corrections necessary for temperature fluctuations. This sphere had a coefficient of expansion so low that it was considered negligible. Within the range of temperatures prevailing in this experiment, corrections for thermal expansion did not materially change the values on the original chart.

A limitation of a more serious nature became evident as the study progressed. The pressure of the tape on the apple, exerted by the small springs in the connected lever mechanism, although very light, appeared to retard fruit enlargement slightly. To correct for this apparent error, each time the chart was changed the calibration of the instrument was checked by measuring the apple with a hand tape. This tape was graduated to millimeters but with care could

be read to one-half millimeter. To further check the relative growth rate, 25 apples on the same tree, selected for uniformity of size, were measured daily with the tape. It was found that the average daily enlargement of these apples followed closely that of the apple in the instrument. The apparatus proved quite sensitive to changes

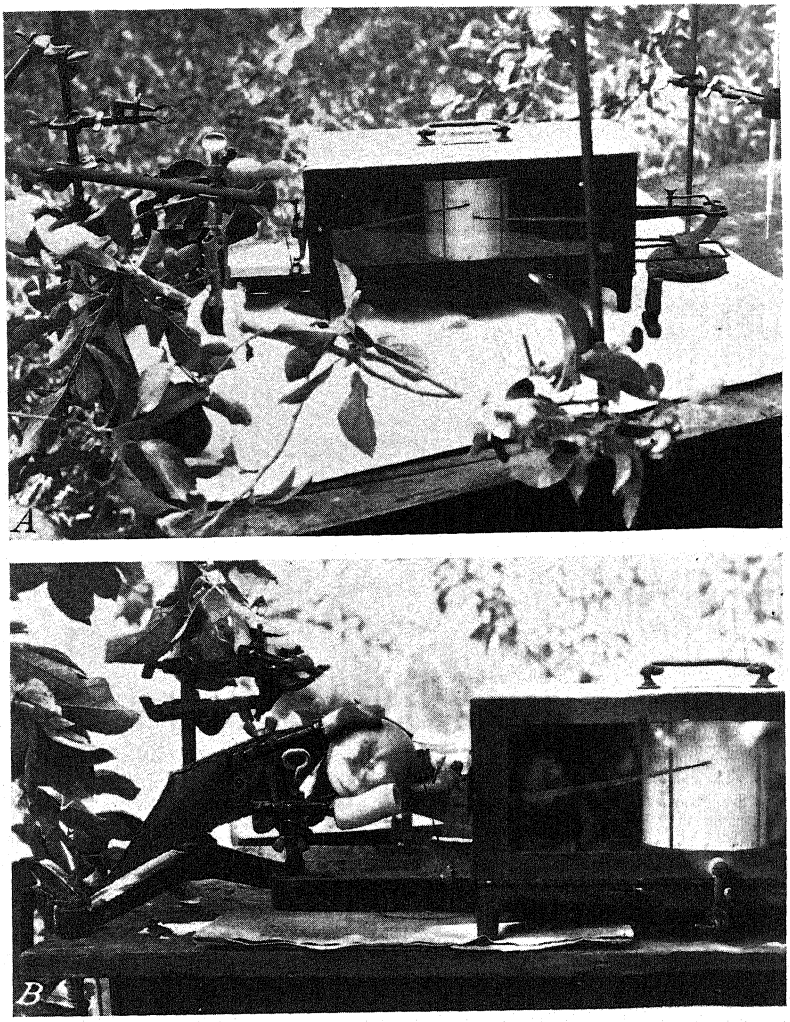


FIGURE 1.—Apparatus used for obtaining a continuous record of growth of apple fruit on the tree: *A*, Clamps holding branches; *B*, apple in position, encircled by brass tape attached to recording mechanism.

in fruit enlargement. A space equivalent to 1° on the 1072-D thermograph chart used represented an average volume increase in the apple of from 0.05 to 0.10 cc, depending on the calibrations of the instrument. In the conversion of circumference measurements to volume, the apple was assumed to be a sphere.

The experiment was carried on from July 20, after extension growth had ceased, until harvest on October 11. With the aid of the instruments a continuous record of fruit growth was obtained during this period. Atmospheric temperatures were recorded simultaneously on the same chart. The relative evaporating power of the air was estimated by the use of standardized white atmometers. One atmometer was read at 4-hour intervals beginning at 8 a. m. and ending at 8 p. m.; the other was read daily at 4 p. m. Air movement was measured by means of a small fan-type anemometer, mounted with a V-type vane and balanced on an enclosed ball bearing. Its response to slight changes in wind direction was rapid and positive. Anemometer and atmometer readings were taken at the same time.

The trees on which the experiment was performed were large, vigorous Winesaps about 30 years of age, carrying a heavy load of fruit. They were growing in a deep, moderately light clay-loam soil with excellent drainage. The soil was irrigated frequently to insure a high moisture supply at all times. The moisture percentages at three soil horizons, with their respective wilting percentages and dates of irrigation, are given in table 1.

TABLE 1.—*Soil-moisture determinations on experimental plot for study of relation of growth of Winesap apples to evaporating power of the air, air movement, and atmospheric temperature, 1936* [1]

Sampling date	Moisture in soil horizon [2]			Sampling date	Moisture in soil horizon [2]		
	1 and 2 feet	3 and 4 feet	5 and 6 feet		1 and 2 feet	3 and 4 feet	5 and 6 feet
	Percent	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*
July 28	19.2	12.5	9.5	Aug. 29	19.3	13.5	12.2
Aug. 1	13.8	11.0	13.2	Sept. 2		12.2	10.5
4	16.4	9.6	12.8	7	17.0	11.7	9.5
8	18.5	12.9	14.8	11	14.9	12.7	14.4
12	19.8	11.6	11.7	18	17.7	12.9	11.8
17	16.0	12.2	10.3	23	13.9	10.2	9.5
21	15.2	12.1	11.1	28	16.8	12.5	11.0
25	14.1	11.5	10.4	Oct. 5	13.9	10.2	11.0

[1] Irrigations: July 24–27; Aug. 5–7, 26–28; and Sept. 15–17.
[2] Wilting points, from laboratory determinations with sunflower plants: 1 and 2 feet, 7.8 percent; 3 and 4 feet, 6.1 percent; 5 and 6 feet, 5.5 percent.

EXPERIMENTAL RESULTS

Examination of the chart records gave early indications of a definite daily periodicity in the enlargement of apples. The rhythm of growth from day to day, however, was not always uniform, nor did it remain the same throughout the season. During the first 4-week period, July 20 to August 16, on clear, bright days, enlargement of the fruit took place almost entirely between 7 p. m. and 10 a. m., the maximum rate occurring between midnight and 2 a. m. This is illustrated by figure 2, which shows that on August 4, 5, and 6 the apples made no perceptible growth between 10 a. m. and 7 p. m.; in fact, the August 6 record indicates very slight shrinkage of the fruit between 3 and 7 p. m. This shrinkage usually was found to occur on days when the evaporating power of the air was relatively high. The loss of water from white atmometers on August 4, 5, and 6 was 26.3, 36.9, and 63.8 g, respectively. Corresponding volume increases of the fruit were

2.03, 1.68, and 1.52 cc. Although the maximum temperatures for August 4 and 5 were considerably higher than that of August 6, the evaporating power of the air was lower, indicating that high evaporating power of the air exerts a greater retarding influence on fruit growth than high temperatures alone, although the two are often closely associated. Reference is also made to table 1, indicating that the soil moisture was not limiting at this time. Water was applied to the soil from August 5 to 7. The fact that the enlargement rate of the fruit was greater before than during irrigation suggests that in this experiment reduction in growth was caused by factors other than lack of available soil moisture.

The August 2 growth curve, which is characteristic of fruit enlargement on days of extremely low evaporating power, is shown in figure 3.

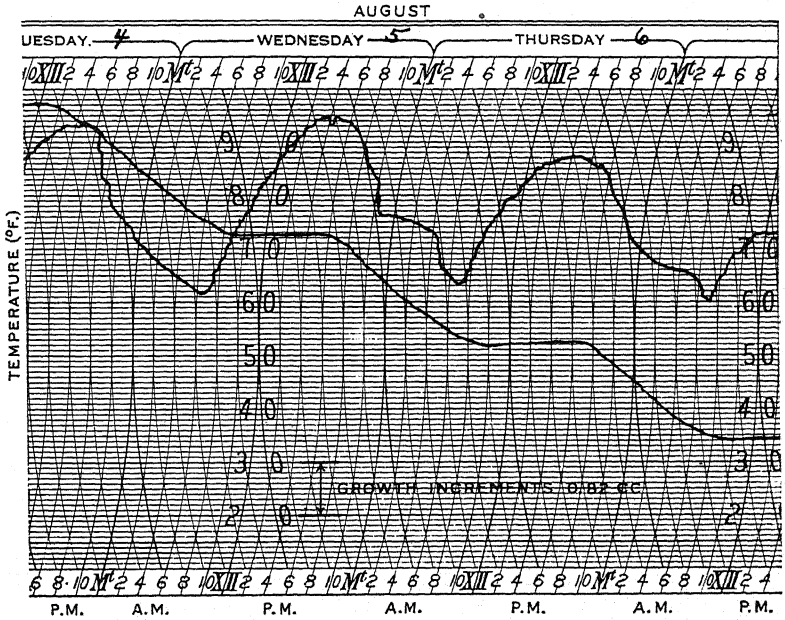


FIGURE 2.—Fruit-growth chart showing periodicity in time and rate of growth of an apple on August 4, 5, and 6. The growth curve is the light line taking a course toward the bottom of the chart. Temperatures are shown by the heavy line. For hours of growth, read bottom; for hours of temperature, read top, deducting 2 hours.

There was little perceptible air movement throughout the day, and the 24-hour water loss from white atmometers was only 18.9 g; the volume increase of the fruit was 2.22 cc. As shown on the chart, fruit enlargement was continuous throughout the 24 hours under these conditions, although the rate was somewhat less during the day than it was at night.

As the season progressed it became quite evident that a change was taking place in the diurnal periodic cycle of growth. In the second 4 weeks of the experiment, August 17 to September 13, the average growth rate and the total amount of growth per day were less than in the previous 4 weeks, and the apples tended to make a slightly greater percentage of their growth during the daylight hours. A

typical curve from the instrument chart illustrating growth during this period is given for August 20 and 21 in figure 4.

Figure 5 shows the growth curves for September 23 and 24, which characterize in general the daily periodicity for the third 4-week period, September 14 to October 11. It is readily observed that the total growth on these days is considerably less than is shown in figures 2, 3, and 4, and also that a different periodicity is exhibited. The period of most rapid enlargement occurred during the morning daylight hours, reaching a maximum between 10 a. m. and 12 noon. In general, during the entire 4 weeks there was a tendency to show some enlargement throughout the 24 hours; however, a fairly well-established minimum prevailed from 4 p. m. to 12 midnight. The average total growth is considerably less than that of the two previous periods.

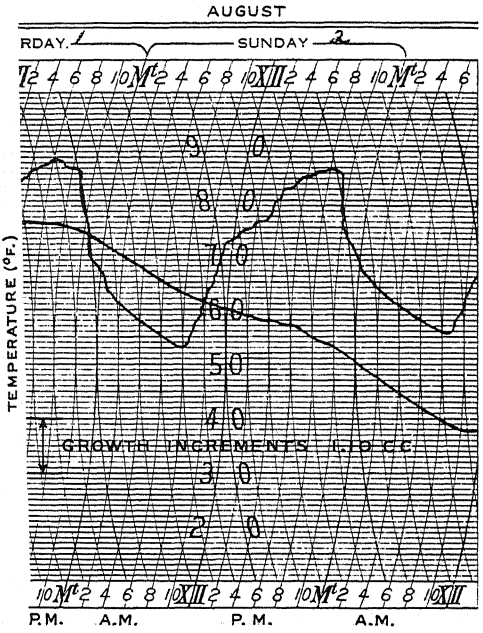


FIGURE 3.—Fruit-growth chart showing periodicity in time and rate of growth of an apple on August 1 and 2. The growth curve is the light line, and temperatures are shown by the heavy line. For hours of growth, read bottom; for hours of temperature, read top, deducting 2 hours.

Variations in average diurnal rate of enlargement of apples in each of the three seasonal divisions are diagrammatically summarized in figure 6. These divisions or periods were arbitrarily selected to demonstrate best the periodic drift over the 3 months' duration of growth. Rate of growth is given in terms of volume increase in cubic centimeters, at 2-hour intervals. The average enlargements per day for the three periods are as follows: July 20 to August 16, 1.89 cc; August 17 to September 13, 1.66 cc; September 14 to October 11, 1.16 cc.

Perhaps the most striking feature of these curves is the shift in time and magnitude of the growth climacterics as the fruit advances toward maturity. The greatest contrasts are found between the first

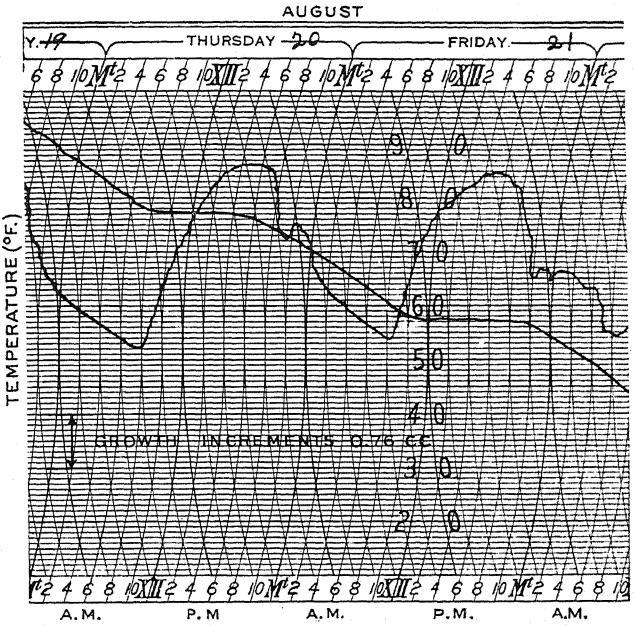


FIGURE 4.—Fruit-growth chart showing periodicity in time and rate of growth of an apple on August 19, 20, and 21. The growth curve is the light line, and temperatures are shown by the heavy line. For hours of growth, read bottom; for hours of temperature, read top, deducting 2 hours.

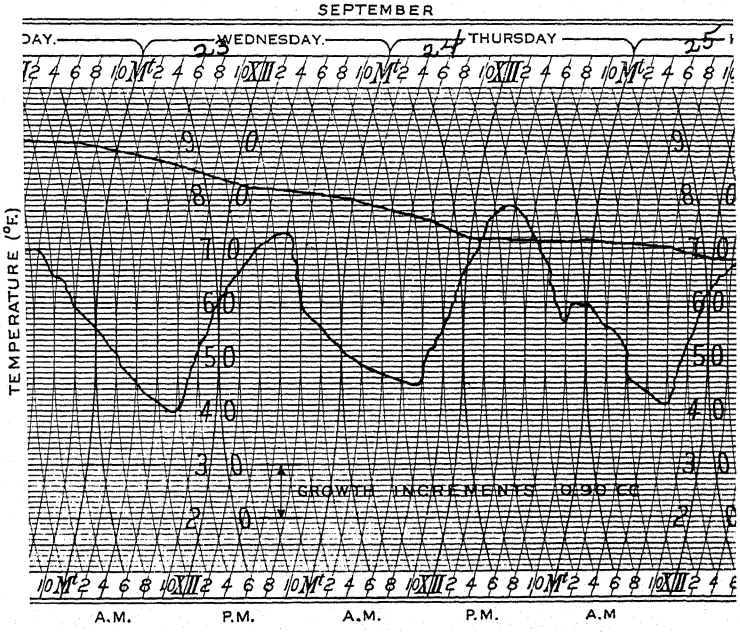


FIGURE 5.—Fruit-growth chart showing periodicity in time and rate of growth of an apple on September 23 and 24. The growth curve is the light line, and temperatures are shown by the heavy line. For hours of growth, read bottom; for hours of temperature, read top, deducting 2 hours.

and third periods. For example, rapid enlargement in the first period takes place within the same hours as does minimum enlargement in the third. Likewise, the hours of maximum growth in the third period occur within the time range of minimum growth in the first.

A relationship perhaps worthy of special emphasis is indicated on this chart (fig. 6). Throughout the 24 hours only two points on each of the three curves coincide in showing both a uniform and a minimum growth. These are found at 4 and 6 p. m. and represent the average lowest rates of enlargement over the three periods. This time period may therefore be considered as having a rather well-established minimum growth rate for this portion of the growing season and might be designated as the "mean diurnal minimum."

The average seasonal enlargement of the 25 hand-measured apples used as a check on the apple in the instrument is shown in figure 7.

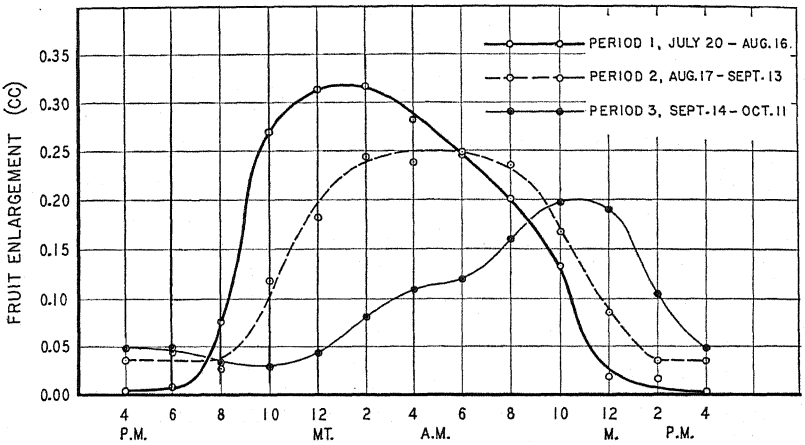


FIGURE 6.—Average diurnal enlargement rate of Winesap apples during the 1936 season, showing change in daily periodicity for three consecutive periods.

This curve shows a departure from straight-line growth early in September, when a decline of approximately 22 percent took place. This deviation from a uniform enlargement may be characteristic of late varieties such as Winesap. Oldenburg (*12*), an early-maturing variety, did not show this change in growth up to the time of harvest.

Attention is again directed to the effect of evaporating power of the air on the daily growth rate and periodicity of fruit enlargement, as mentioned above and as illustrated in figures 2 and 3. In table 2 the average daily enlargement of fruit, mean temperature, and air movement, occurring with different rates of evaporating power of the air, are given for the three 4-week periods in 1936. In preparing this table the days of each period were arbitrarily divided into four groups according to the evaporating power of the air, and each day's growth, mean temperature, and air movement were placed in their respective evaporation groups and averaged. A very definite negative correlation can be seen between average daily fruit growth and evaporating power of the air. Of the factors studied, including evaporating power, air movement is apparently an especially important one and follows closely the same relationship to growth in the last two periods but not in the first. The deduction, therefore, is that in this experiment air

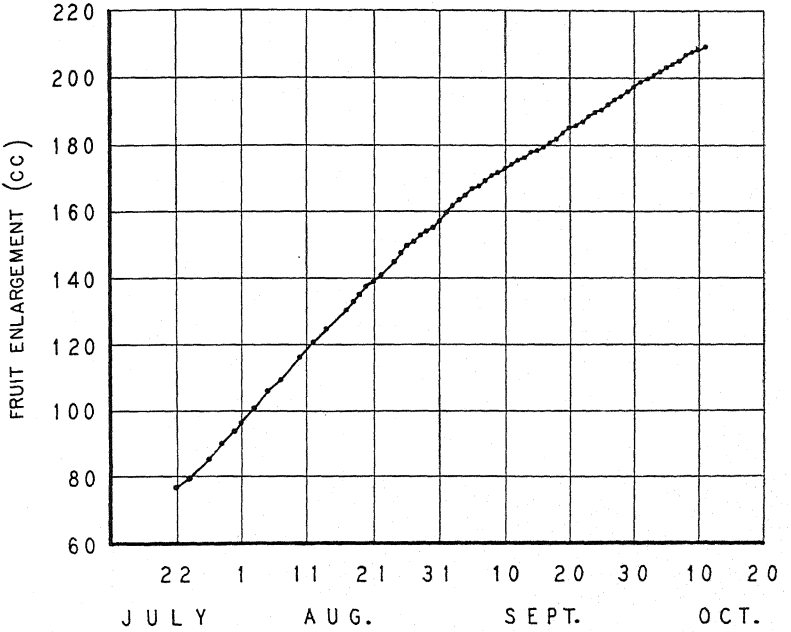


FIGURE 7.—Average seasonal enlargement of 25 Winesap apples obtained from hand-tape measurements.

TABLE 2.—*Relation of average daily enlargement of Winesap apples, air movement, and mean temperature, to four levels of evaporating power of the air, 1936*

Evaporating power of air (grams H_2O per day)	First period (July 20 to Aug. 16)			Second period (Aug. 17 to Sept. 13)			Third period (Sept. 14 to Oct. 11)		
	Enlargement of fruit per day	Linear air movement per day	Mean temperature	Enlargement of fruit per day	Linear air movement per day	Mean temperature	Enlargement of fruit perday	Linear air movement per day	Mean temperature
	Cc	*Feet* [1]	° *F.*	*Cc*	*Feet* [1]	° *F.*	*Cc*	*Feet* [1]	° *F.*
40 and above	1.79	207	75.4	1.42	212	66.1			
30 to 40	1.99	104	72.6	1.49	148	63.8	0.83	165	61.7
20 to 30	2.15	39	74.7	1.77	41	65.3	1.17	114	59.0
Less than 20				1.93	59	61.3	1.23	43	54.1

[1] In thousands.

movement was probably the dominating factor of those affecting the daily enlargement rate of apples. The correlation coefficients for fruit enlargement, evaporating power of the air, air movement, and temperature are shown in table 3.

The possible association of atmospheric influences with daily periodicity of fruit enlargement is indicated in table 4. Here the 24-hour day is divided into four intervals, and corresponding average hourly growth rates, mean temperatures, evaporating power of the air, and air movements are given for comparison. Examination of these data reveals that, with the exception of the 4 p. m. to 8 a. m. interval in the third period, the hours of relatively high growth rate are invariably associated with either low evaporating power of the air or low air

movement, or both; conversely, hours of low growth are associated with either high evaporating power or increased air movement, or both. This indicates a relationship between growth rate and evaporating power for divisions of the day similar to that between average total daily growth and evaporating power as shown in tables 2 and 3. It is rather significant that the lowest fruit-growth rates occur between 12 noon and 8 p. m., and that the highest evaporating powers of the air and air movements also occur during this time. The fact that the greatest movement of air takes place in the latter half of this period, between 4 p. m. and 8 p. m., is noteworthy, for it is within these hours that the mean diurnal minimum evaporation is found.

TABLE 3.—*Correlation coefficients for fruit enlargement, evaporating power of air, air movement, and temperature (from data in table 2)*

Item	Evaporating power of air		Air movement		Temperature	
	r	*P*[1]	*r*	*P*[1]	*r*	*P*[1]
Fruit enlargement	−0.95	<0.01	−0.91	<0.01	−0.76	0.01
Evaporating power of the air			+.97	<.01	+.65	.03

[1] Fisher's "probability" (*z* test used) (*11*).

TABLE 4.—*Relation of average hourly enlargement of Winesap apples, evaporating power of the air, air movement, and mean temperature, to four time periods of 24-hour day, 1936*

Time intervals	First period (July 20 to Aug. 16)				Second period (Aug. 17 to Sept. 13)				Third period (Sept. 14 to Oct. 11)			
	Enlargement of fruit per hour	Evaporating power of air (H_2O per hour)	Linear air movement per hour	Mean temperature	Enlargement of fruit per hour	Evaporating power of air (H_2O per hour)	Linear air movement per hour	Mean temperature	Enlargement of fruit per hour	Evaporating power of air (H_2O per hour)	Linear air movement per hour	Mean temperature
	Cc	*Grams*	*Feet*	° *F.*	*Cc*	*Grams*	*Feet*	° *F.*	*Cc*	*Grams*	*Feet*	° *F.*
8 a. m. to 12 m.	0.038	2.48	6,500	81.0	0.063	1.82	5,300	71.0	0.097	1.00	2,700	65.3
12 m. to 4 p. m.	.005	3.08	5,900	86.4	.018	2.50	7,000	76.9	.038	2.20	5,300	70.1
4 to 8 p. m.	.021	2.32	7,300	81.1	.018	1.75	7,500	67.4	.021	1.20	6,400	58.9
8 p. m. to 8 a. m.	.136	.82	4,200	65.8	.105	.43	2,700	55.5	.045	.13	1,100	47.9

Average temperatures throughout the 24-hour day for each of the three periods of the experiment are shown in figure 8. Approximate hours and temperatures of maximum and minimum rate of fruit enlargement are also indicated. That temperature may be a factor in the changes in seasonal periodicity is evidenced by this chart. It appears significant that the hours of maximum fruit growth in all periods occur within the 50° to 75° F. range regardless of the hour of day or night when this high growth rate occurs. The possible relation of temperature to hours of minimum growth is not so apparent, however. On the curve for July 20 to August 16, minimum enlargement rate is associated with temperatures well above 80°. From August 17 to September 13, or during the second period, the minimum rate broke into temperatures occurring at the time of maximum growth in the first period; while in the final period, September 14 to October

11, both maximum- and minimum-growth hours fell largely within the same temperature limits. This does not necessarily show that low temperatures may not retard fruit enlargement, for in figure 6 the curve representing the period from September 14 to October 11 clearly indicates a retardation in rate of growth during the night and early morning hours when temperature is comparatively low, especially between 4 and 6 a. m. Data from this experiment, however, give no evidence that temperature alone has any pronounced effect on the mean diurnal minimum.

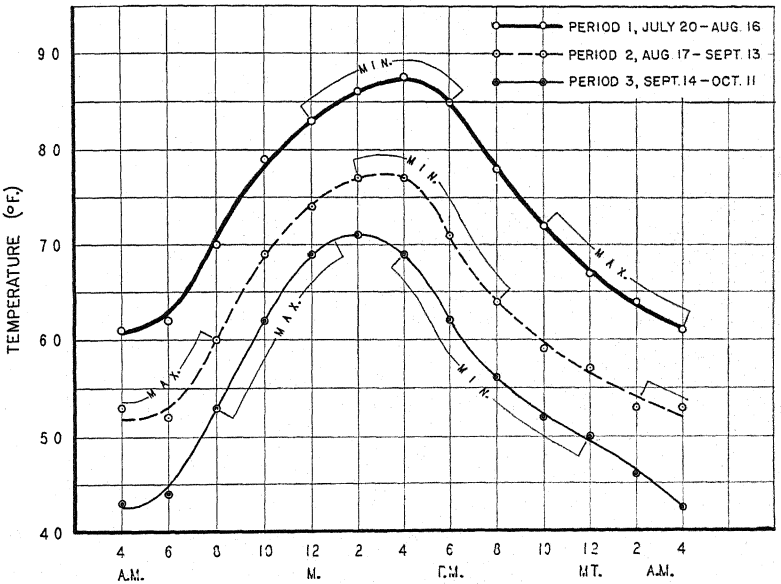


FIGURE 8.—Average atmospheric temperatures through 24-hour day during 1936 season, and approximate hours of maximum and minimum fruit-enlargement rate for three consecutive periods.

Temperature relationships, in contrast with evaporating power and air movement, seem again to be uncertain, and it appears that within the temperature limits of this experiment high temperature exerts an indirect effect on growth of fruit through increasing the evaporating power of the air. That low temperatures may directly limit growth is evidenced in the third period from 8 p. m. to 8 a. m. (table 4). This was the only time during the experimental season that maximum growth was not associated with minimum evaporating power and air movement. It was also the only time that the average temperature fell below the optimum growth range of 50° to 75° F. (fig. 8). Apparently, then, temperature below a certain range is a limiting factor in fruit enlargement, and this may account for the shift in the hours of maximum enlargement in the third period as compared with the first and second (fig. 6).

DISCUSSION

The results reported in this paper are too limited to enable one to generalize with much confidence on the many processes involved in plant periodicity, but they do point to a few environmental factors that seem to be of importance in regulating the periodic enlargement of apples. In this investigation, evidence presented indicates that time and relative rate of fruit enlargement are closely associated with atmospheric conditions. Aldrich and Work (*1*) found that pears showed a reduced growth rate during days of high transpiration, when soil moisture was highly available. Data from the present experiment (figs. 2 and 3; table 2) substantiate these findings and in addition to this reduction of total daily growth by high evaporation show a definite association between the hours of the day when the evaporating power of the air was highest and the rate of growth of the fruit was lowest (table 4).

If one considers that enlargement of apples depends on the transport of water and elaborated substances to the fruit from other portions of the tree, any factor or group of factors interfering with this movement would probably retard enlargement. A high evaporating power of the air would tend to increase water loss from the leaves, and it is thus possible that, during periods of high transpiration accompanied by a saturation deficit in the leaf, transport of materials to the fruit may be retarded and water may actually be withdrawn from the fruit. Upon the restoration of leaf turgor, which usually occurs at night during the summer, a resumption of fruit enlargement takes place.

It is not within the scope of this report to discuss the effect on transpiration or translocation of each individual factor that goes to make up the evaporating power of the air. However, under the conditions of this investigation there is rather conclusive evidence that air movement was the most important single factor studied in increasing the evaporating power of the air, as measured by water loss from white atmometers. If an attempt is made to account for increased transpiration in terms of air movement the writers are not wholly in agreement with the findings of many investigators; however, the experimental evidence in regard to the influence of wind on transpiration and plant response is itself somewhat confusing. Briggs and Shantz (*4*, *5*) found that, of the physical environmental factors studied, wind velocity showed the lowest correlation with transpiration. They considered that only 2 to 6 percent of the transpirational loss could be attributed to the action of wind.

The apparently conflicting evidence regarding factors that affect transpiration in the field may in some cases be traced to climatic differences in the location of the experiment. It is possible that under certain atmospheric conditions air movement, as compared with other influences, may have but a slight effect in increasing transpiration. Studies in the field, at best, are complicated by many opposing and interacting factors and the interpretations frequently reflect only the forces most active at a particular time.

The terrain adjacent to the orchards of the Wenatchee Valley is arid during the summer months. In the morning and early afternoon the surrounding desert land and rocky hillsides absorb considerable heat from the sun's rays. Subsequent air movement in

the afternoon tends to carry into the cooler orchards the resultant hot, dry atmosphere from these outside areas. Under these conditions air movement, in addition to exerting a possible direct influence in increasing water loss from the leaves, may also produce the same result by bringing about a combination of forces, such as lowered humidity and increased atmospheric and leaf temperatures.

Knight (*17*) found that a wind velocity of 0.26 mile per hour caused a 50-percent increase in transpiration rate over that in still air. Copeland (*8*) in the Philippine Islands found that transpiration of the coconut palm was four times as great in a wind with a velocity of 5 miles per hour as in a calm. Others (*6, 19, 21*), however, have concluded that transpiration was increased up to a limited air velocity, above which there was a pronounced depressing effect. Martin and Clements (*19*), using motor-driven fans on sunflowers, found a striking decrease in leaf and stem development, dry weight, and total transpiration with increasing wind velocities. These findings suggest that air movement may bring about physiological changes, correlated with increased transpiration, which may in themselves exert a retarding influence on fruit enlargement.

Recently Audus (*2*), and also Godwin (*13*), studying the effect of various treatments on the respiration rate of leaves, reported a twofold to threefold increase in respiration resulting merely from bending or rubbing the leaves lightly past one another. After the treatment, respiration gradually decreased, but did not reach normal until after about 60 hours. From the results obtained by these investigators it appears that respiration of apple leaves may be increased by the motion or rubbing action caused by wind to the extent of retarding fruit enlargement. Heinicke and Childers (*14*) offer further evidence in this direction by showing that during the daytime a high rate of respiration in apple leaves greatly reduced the rate of photosynthesis.

These possibilities, either singly or collectively, point to a partial explanation of the effects of air movement on fruit growth. They also suggest that a reduction in diurnal fruit enlargement may not be due entirely to a temporary delay in translocation, but that some of the elaborated materials may be permanently lost to the fruit. It has been observed that apple trees in localities characterized by constant winds are usually stunted in growth and seldom highly productive. Blanchard (*3*) found a 5- to 7-percent increase in yield of lemons and a 3-percent increase in size of tree when the grove was protected from wind.

If the enlargement rate of apples through the season is adversely affected by high evaporating power of the air, there would probably be evidence of the same influence on daily periodic growth. Data supporting this assumption are shown in table 4. An important factor to be considered in this connection is the time of day, throughout the season, when fruit-enlargement rate is likely to exhibit the least variability. In the present experiment such a time was found between 4 and 6 p. m., when the average minimum growth rate of apples for the experimental season occurred (fig. 6). Daily fruit measurements taken at this time, therefore, will probably be influenced least by periodic growth variation. In this work this period has been designated as the "mean diurnal minimum." It must be recognized, however, that this may hold true only under conditions in the Pacific Northwest or perhaps within even narrower geographic limits.

To account fully for the change in diurnal periodicity from one seasonal period to the next is rather difficult with the data at hand. Shifting of the daily maxima of fruit enlargement both in time and magnitude as the fruit approaches maturity is undoubtedly brought about by fundamental changes in metabolism, some of which, as have been shown, are probably influenced by environmental conditions. Other changes might be the result of hereditary characteristics peculiar to the species or variety. That pronounced periodicity can be induced in plants by variation in the amount of daily illumination is well recognized, and this may also play an important part in the seasonal periodicity of fruit enlargement.

During the course of this investigation, for example, daily illumination as measured from sunrise to sunset decreased from 15½ to 11 hours. According to Heinicke and Hoffman (*15*), such a reduction in length of day would decrease photosynthetic activity of the leaf. This, accompanied by lowering light intensities as the season progresses (*7*), might result in a lower rate of fruit growth.

The growth curve for the third period (fig. 6) is of particular interest in the light of recent researches by Curtis and Herty (*10*) on the effect of relatively low temperatures on translocation. These authors found that chilling the petioles of bean leaves to temperatures between 0.5° and 4.5° C. greatly reduced transport of carbohydrates from the leaves, and that maintaining the petioles at 7° to 11° allowed greater translocation, but less than at temperatures of 17° to 24°. The fruit-enlargement curve for this third period shows a response to temperature variations similar to that described above. The relatively low average night temperatures of this period (6.5° to 10°) fall within one of the temperature ranges used by Curtis and Herty (*10*), and it appears significant that the hours of slow fruit enlargement coincide with these comparatively low temperatures. The rather sharp change in slope of the curve (fig. 6) from 4 to 6 a. m., where the lowest average temperatures were encountered, tends to emphasize this relationship.

SUMMARY AND CONCLUSIONS

By means of continuous-recording instruments, checked by daily hand-tape measurements, a study was made of the relationship between atmospheric conditions and the time and rate of enlargement of Winesap apples.

Reduction in the enlargement rate of fruit was closely associated with comparatively high evaporating power of the air, and under the conditions of this investigation air movement appeared to be the dominant factor in increasing this evaporating power.

Atmospheric temperatures probably have an important influence on the enlargement rate of apples. The effect of high temperatures in retarding growth may be exerted indirectly by increasing the evaporating power of the air. There is some indication that temperatures below 50° F. may directly limit fruit growth.

A pronounced daily periodicity was found in the time and rate of fruit enlargement, and this periodicity seemed to be influenced by the evaporating power of the air. Hours during which average growth was at a minimum were characterized by high evaporating power of the air and increased air movement.

The daily curve of growth did not remain constant throughout the investigation but showed a distinct seasonal shift in maximum and minimum rates. From July 20 to August 16 the highest rate of fruit enlargement occurred between 10 p. m. and 4 a. m.; from August 16 to September 13 the maximum rate was between 2 and 8 a. m.; while in the third period, September 14 to October 11, it was found from 8 a. m. to 1 p. m. The average daily growth for these three periods was 1.89, 1.66, and 1.16 cc, respectively.

The least fluctuation in daily growth rate throughout the experiment was found in the minimum growth range, between 4 and 6 p. m. This period has been termed the "mean diurnal minimum" of fruit enlargement. Daily fruit measurements taken at this time will therefore be less affected by both diurnal and seasonal periodic variations in growth than at any other time of the 24 hours.

Some possible physiological effects of air movement and evaporating power of the air on the retardation of fruit enlargement are discussed.

LITERATURE CITED

(1) ALDRICH, W. W., and WORK, R. A.
1935. EVAPORATING POWER OF THE AIR AND TOP-ROOT RATIO IN RELATION TO RATE OF PEAR FRUIT ENLARGEMENT. Amer. Soc. Hort. Sci. Proc. (1934) 31 (v. 32): 115–123, illus.

(2) AUDUS, L. J.
1935. MECHANICAL STIMULATION AND RESPIRATION RATE IN THE CHERRY LAUREL. New Phytol. 34: 386–402, illus.

(3) BLANCHARD, VINCENT F.
1934. DEPRESSING EFFECTS OF WIND ON GROWTH AND YIELD OF CITRUS TREES. Calif. Citrogr. 19: 206, illus.

(4) BRIGGS, LYMAN J., and SHANTZ, H. L.
1916. HOURLY TRANSPIRATION RATE ON CLEAR DAYS AS DETERMINED BY CYCLIC ENVIRONMENTAL FACTORS. Jour. Agr. Research 5: 583–650, illus.

(5) ——— and SHANTZ, H. L.
1916. DAILY TRANSPIRATION DURING THE NORMAL GROWTH PERIOD AND ITS CORRELATION WITH THE WEATHER. Jour. Agr. Research 7: 155–212, illus.

(6) BROWN, MAUDE A.
1910. THE INFLUENCE OF AIR-CURRENTS ON TRANSPIRATION. Iowa Acad. Sci. Proc. 17: 13–15.

(7) CHRISTOPHER, E. P.
1935. THE INTENSITY OF LIGHT STRIKING LEAVES OF APPLE TREES AT DIFFERENT TIMES OF DAY. Amer. Soc. Hort. Sci. Proc. (1934) 31 (v. 32): 86–92, illus.

(8) COPELAND, EDWIN BINGHAM.
1906. ON THE WATER RELATIONS OF THE COCOANUT PALM (COCOS NUCIFERA). Philippine Jour. Sci. 1: 6–57, illus.

(9) CULLINAN, F. P., and WEINBERGER, J. H.
1933. STUDIES ON THE INFLUENCE OF SOIL MOISTURE ON GROWTH OF FRUIT AND STOMATAL BEHAVIOR OF ELBERTA PEACHES. Amer. Soc. Hort. Sci. Proc. (1932) 29: 28–33, illus.

(10) CURTIS, OTIS F., and HERTY, S. DOROTHEA.
1936. THE EFFECT OF TEMPERATURE ON TRANSLOCATION FROM LEAVES. Amer. Jour. Bot. 23: 528–532.

(11) FISHER, RONALD AYLMER.
1930. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 3, rev. and enl., 283 pp., illus. Edinburgh and London.

(12) FURR, J. R., and MAGNESS, J. R.
1931. PRELIMINARY REPORT ON RELATION OF SOIL MOISTURE TO STOMATAL ACTIVITY AND FRUIT GROWTH OF APPLES. Amer. Soc. Hort. Sci. Proc. (1930) 27: 212–218, illus.

(13) GODWIN, H.
1935. THE EFFECT OF HANDLING ON THE RESPIRATION OF CHERRY LAUREL LEAVES. New Phytol. 34: 403–406, illus.
(14) HEINICKE, A. J., and CHILDERS, N. F.
1937. INFLUENCE OF RESPIRATION ON THE DAILY RATE OF PHOTOSYNTHESIS OF ENTIRE APPLE TREES. Amer. Soc. Hort. Sci. Proc. (1936) 33 (v. 34): 142–144.
(15) ——— and HOFFMAN, M. B.
1933. THE RATE OF PHOTOSYNTHESIS OF APPLE LEAVES UNDER NATURAL CONDITIONS. PART I. N. Y. (Cornell) Agr. Expt. Sta. Bull. 577, 32 pp., illus.
(16) HENDRICKSON, A. H., and VEIHMEYER, F. J.
1929. IRRIGATION EXPERIMENTS WITH PEACHES IN CALIFORNIA. Calif. Expt. Sta. Bul. 479: 3–56, illus.
(17) KNIGHT, R. C.
1917. RELATIVE TRANSPIRATION AS A MEASURE OF THE INTRINSIC TRANSPIRING POWER OF THE PLANT. Ann. Bot. [London] 31: 351–359.
(18) MACMILLAN, CONWAY.
1891. ON THE GROWTH-PERIODICITY OF THE POTATO TUBER. Amer. Nat. 25: 462–469.
(19) MARTIN, E. V., and CLEMENTS, F. E.
1935. STUDIES OF THE EFFECT OF ARTIFICIAL WIND ON GROWTH AND TRANSPIRATION IN HELIANTHUS ANNUUS. Plant Physiol. 10: 613–636, illus.
(20) SACHS, JULIUS.
1874. UEBER DEN EINFLUSS DER LUFTTEMPERATUR UND DES TAGESLICHTS AUF DIE STÜNDLICHEN UND TÄGLICHEN AENDERUNGEN DES LÄNGENWACHSTHUMS (STRECKUNG) DER INTERNODIEN. Arb. Bot. Inst. Würzburg v. 1, pp. 99–192.
(21) WILSON, H. W.
1924. STUDIES ON THE TRANSPIRATION OF SOME AUSTRALIAN PLANTS, WITH NOTES ON THE STRUCTURE OF THEIR LEAVES. Roy. Soc. Victoria Proc. 36: [175]–237, illus.

THE SOLUBILITY OF CASEIN IN SALTS OF CERTAIN ORGANIC ACIDS, AND ITS FRACTIONATION BY MEANS OF THESE ACIDS [1]

By RALPH H. MCKEE, *professor of chemical engineering, Columbia University,* and STEPHEN P. GOULD, *assistant chemist, Division of Dairy Research Laboratories, Bureau of Dairy Industry, United States Department of Agriculture* [2]

INTRODUCTION

The study of casein, the principal protein in milk, is being given increasing attention in research laboratories, not only because of the promising new ways in which it may be used in industry, but also because it affords a suitable material for the study of fundamental problems in protein chemistry.

Strong acid and alkaline reagents have generally been used as solvents for casein, but they are not entirely satisfactory. Accordingly, the writers undertook experiments to determine the degree of solubility of casein in solutions of certain salts of organic acids. Nearly neutral aqueous solutions having a pH value of 4.6 (the isoelectric point of casein) were used in these experiments, since very little research work had been done with such solutions as compared to the work done with strong acids and bases.

DEFINITION OF TERMS

The term "casein" as used here means "acid casein" as distinct from rennet casein. Acid casein might be further defined as the phosphoprotein, or aggregation of phosphoproteins, precipitated from skim milk by acid at a pH value of 4.1 to 4.6. The phosphorus content, as determined by different investigators, varies from 0.71 to 0.88 percent.

Rennet casein is evidently a calcium caseinate—calcium phosphate complex—and is not nearly so pure as acid-precipitated casein. Rennet casein is relatively unimportant commercially; approximately 97 percent of all the casein produced is acid casein. Acid casein is the product commonly known in the industry and in the scientific literature as "casein."

By "solubility of casein" is meant the solubility at the isoelectric point rather than that resulting from chemical combination, as, for example, in borax solution where sodium caseinate is formed and dissolved.

The term "solution" in this paper refers both to crystalloidal and to colloidal solutions.

[1] Received for publication January 29, 1938; issued August 1938. This paper was presented in part before the meeting of the American Chemical Society, Chapel Hill, N. C., April 1937. It is condensed from the dissertation submitted by Stephen P. Gould in partial fulfillment of the requirements for the degree of doctor of philosophy in the Faculty of Pure Science, Columbia University.

[2] The writers acknowledge the generous cooperation and helpful advice of L. A. Rogers, Chief, and E. O. Whittier, senior chemist, Division of Dairy Research Laboratories, Bureau of Dairy Industry.

SOLUBILITY OF ACID-PRECIPITATED CASEIN

EARLIER INVESTIGATIONS DEALING WITH SOLUBILITY OF CASEIN

Much of the earlier work on casein solvents or plasticizers in the organic field was done in connection with casein plastics. Goldsmith (*12*, *13*, *14*) [3] in 1907 found that naphthol and other phenolic compounds and urea would "convert" casein to the "thermoplastic" state. This he believed was due to "the solvent action of the converting agent." Phenolsulphonic acid, tartaric acid, aniline, borax, and naphtholsulphonic acid (*28*) are also mentioned in the early patent literature of plastic casein.

Cohn (*7*, *8*) reported the solubility of casein in water at its isoelectric point as 0.11 g per liter at 25° C. Euler and Bucht (*9*) determined quantitatively the solubility of casein in tartaric acid, sodium potassium tartrate, and chloracetic acid solutions. They determined the amount of casein in the solution by precipitating it with sodium acetate solution or acetic acid, filtering, and drying in a vacuum desiccator. At the Carlsberg Laboratories in 1925 Kondo (*19*) examined the effect of hydrochloric acid on casein. By formol titration, he showed that there was no hydrolysis at 18° C.

Agthe (*1*) records citric acid, sodium naphthylaminosulphonate, and potassium butyrate as agents for making casein soluble.

In view of the fact that an acid that considerably swells casein will usually dissolve it, the work of Isgarischew and Pomeranzewa (*16*) is of great interest. They tested the swelling action of casein in solutions of 23 organic acids. The greatest swelling action was shown by chloracetic, lactic, glycollic, malonic, formic, and citric acids (in the order named). They concluded that a hydroxyl and also a second carboxyl group in the acids greatly increased the swelling of the casein, but that a further increase in carboxyl groups diminished dilation. With the exception of formic acid, the monobasic fatty acids showed little swelling power.

Sutermeister (*31*, *p. 128*) lists a wide variety of solvents for casein, chiefly inorganic. In addition to the commonly used alkalies, he records less commonly used alkaline solvents such as sodium sulphite, aluminite, arsenate, and citrate, and lithium, magnesium, and cesium hydroxides.

Von Weimarn (*36*, *37*) formed dispersions of nitrogenous materials in solutions of $Ca(CNS)_2$, $CaBr_2$, and LiCNS. The last was by far the most efficient solvent. He observed also that solutions of pyrogallol, resorcinol, thiourea, and guanidine thiocyanate were good dispersing agents—particularly at 108° C.

In a fundamental study of organic solvents for proteins, Loiseleur (*22*, *23*, *24*) pointed out that they formed true solutions in certain acids, such as formic, while higher acids, such as acetic and propionic, did not bring about solution. However, oxypropionic acids such as lactic or pyruvic readily dissolved casein. Other acids, such as acetic, that did not have the power of dissolving protein acquired this property when amino acids, such as glycocoll or alanine, were added.

In 1932, Soff (*29*) at Columbia University worked with new types of solvents developed by the senior author. These are concentrated aqueous solutions of highly soluble salts of organic acids such as

[3] Italic numbers in parentheses refer to Literature Cited, p. 141.

sodium cymenesulphonate or sodium benzenesulphonate in which the organic part is a large proportion of the molecule. Such solutions differ from water in their solvent characteristics, as the organic portion of the compounds seems to give them many of the properties of organic solvents. In general, Soff worked with highly concentrated solutions. He found sodium cymenesulphonate solution to be a very powerful solvent for casein and gelatin. He also stated; "There had apparently been no change in properties during storage of these casein solutions over an unusually long period of time." Nearly neutral solvents such as these salts obviously have a greater advantage in avoiding the factor of hydrolysis over stronger, more commonly used basic or acidic compounds.

Associates of Rogers (*2, p. 53*) explain that casein is insoluble in the usual neutral organic solvents and in cold dilute acids. Casein at its isoelectric point of 4.6 is considered to be uncombined with acids or bases.

EXPERIMENTAL PROCEDURE

Materials

Preliminary experiments were made to determine what concentrations would be practical to use for exact solubility work. For these preliminary experiments, sodium cymenesulphonate from the Columbia University chemical engineering laboratory was used. This was fairly pure, but for the more precise work later, the salt was recrystallized from alcohol.

Solubility determinations were run at a pH of 4.6, the isoelectric point of casein, so that the solubility would not be affected by possible alkalinity of the solvent, and also because casein is most stable at its isoelectric point. To adjust the solutions to this point, cymenesulphonic acid was used. This was purified by freeing it from sulphuric acid by the following procedure. The solution was diluted, neutralized with barium hydroxide, and the barium sulphate was filtered off. The cymenesulphonic acid was liberated from its barium salt in the filtrate by the addition of the calculated amount of sulphuric acid. After the barium sulphate was filtered off, the filtrate was evaporated to dryness.

The casein used for the preliminary experiments was a well-washed grain-curd product prepared at the Grove City (Pa.) Creamery. For the later more exact experiments, a purified casein prepared by E. O. Whittier of the Division of Dairy Research Laboratories was employed. This was precipitated from skim milk by adding a 4-percent solution of hydrochloric acid until the pH value was 4.1. The casein was washed first with water slightly acidified with hydrochloric acid, then 8 or 10 times with pure water, next with alcohol, and finally with ether. Thus the redissolving in alkali (which is a concomitant of the Hammarsten process) was avoided. This gave a casein of high purity, containing less than 0.50 percent ash above that due to the P_2O_5 combined in the casein molecule. Hammarsten casein also was used in some of the later experiments.

Methods

The general procedure for determining the solubility of casein in the solutions of the salts of organic acids was as follows: The quantity

of salt necessary to give the desired concentration of solution was dissolved in water, enough of the acid or alkali (as the case might be) corresponding to the salt used was added to bring the pH value of the solution to 4.6, and water was added to bring the whole mixture up to 55 cc. At first the casein was added gradually, often in amounts greater than 5 g, but it was found (in agreement with earlier work on solutions in other solvents) that the solubility increased with increasing amounts of the solid phase. This fact in itself was one proof of the nonhomogeneity of casein. Because of this characteristic, it was considered advisable in all cases to use the same gross quantity of casein. Consequently, the solubility values in this paper, unless otherwise noted, have been determined on the empirical basis of 5 g of casein to 50 cc of solution.

In some of the earlier experiments, much difficulty was caused by bacterial decomposition of the solutions. The addition of 0.2 percent of phenol (0.10 g) to 50 cc of solution served effectively to inhibit this action, and it was determined by experiment that phenol at the low concentration employed did not appreciably affect the solubility values.

Five grams of casein and 50 cc of solution at a pH value of 4.6 were put into a 250-cc Erlenmeyer flask. This was well stoppered and placed in an incubator that was held at the necessary temperature by thermostatic control. From two to four determinations were made in establishing the solubility value at each temperature and concentration. The flasks were shaken at frequent intervals.

The progress toward solubility equilibrium was followed by obtaining the refractive index of the solution at suitable intervals. Samples were drawn off by vacuum through a short piece of rubber tubing fitted on the end of a pipette. The tubing contained a wad of cotton to serve as a filter. An Abbé refractometer, with prisms heated to 30° C., was employed, and therefore samples of only 1 or 2 drops of solution were required. The attainment of equilibrium was shown by the constancy of the maximum of the refractive indices over a period of from 2 to 4 days. This was, incidentally, evidence against hydrolytic decomposition of the protein.

After the equilibrium of the solution was established, some of it was filtered as quickly as possible through a wad of cotton placed in the stem of a funnel. From 1 to 2 g of the solution was weighed in a weighing bottle, transferred to a beaker, and diluted with water at a temperature of 35° to 40° C. A few cubic centimeters of 10-percent acetic acid solution were then slowly added to the warm solution to precipitate the casein. At 35° to 40°, the protein separates in a granular, easily filterable condition. The mixture was allowed to stand overnight to promote completeness of precipitation. The casein was filtered off on a Gooch crucible fitted with two pads of filter paper. The filtrate was concentrated nearly to dryness on the steam plate and diluted with water. If any casein remained in solution, it was practically all removed by this method. Sometimes small additional quantities of casein were thus obtained and were added to the main residue. The Millon or biuret test was applied to the filtrate and usually showed the absence of significant amounts of casein. The precipitate was washed free of extraneous matter. Tests for completeness of washing were made with litmus paper. In

addition, lead nitrate solution was used to test for sodium cymenesulphonate; ferric chloride solution was used to test for potassium thiocyanate; and silver nitrate solution to test for sodium benzenesulphonate.

Acetic acid was chosen as the casein precipitant because this acid, as well as trichloroacetic, has been used for years for this purpose and appears to have been thoroughly tested. Colloid chemists are of the opinion that the action is a salting-out effect. The action is in accordance with the precipitating characteristics of the Hofmeister series—a satisfactory explanation of which is still lacking. To make sure that the precipitates were free from acetic acid, they were always washed until the wash water was neutral to litmus.

In the early experiments, the quantity of precipitated casein was determined by the refractometer. At times, this procedure seemed to give accurate results, but now and then the results were erratic and the source of the error could not be located.

The direct-weight method was finally adopted as the general method for determining the quantity of casein dissolved in the solvents, as it gave more consistent results than the refractometric procedure, was more expeditious than the determination of nitrogen content, and was sufficiently accurate. In obtaining the direct weight of casein, the precipitate on the suction filter was washed free of salts and treated with 60 to 80 cc of acetone and then with the same quantity of ether. The ether was removed by sucking air through the filter for 15 or 20 minutes. The Gooch crucible containing the washed casein was then placed in a desiccator and allowed to remain overnight. From the weight obtained, the number of grams of casein dissolved in 100 g of solvent was calculated.

Unless otherwise indicated, Whittier's purified casein was used for the final solubility determinations.

SOLUBILITY OF CASEIN IN SOLUTIONS OF RECRYSTALLIZED SODIUM CYMENESULPHONATE

Solutions containing 5 percent and 7.5 percent of recrystallized sodium cymenesulphonate were used since the high viscosities of the more concentrated solutions prevented effective sampling.

In general, the 5 percent solution was prepared as follows: 2.47 g of the salt was dissolved in water. Two cubic centimeters of phenol solution (containing 0.10 g) was added, followed by 4 drops of cymenesulphonic acid solution (containing 0.03 g) which gave the solution a pH value of 4.6, as shown by bromocresol green paper. The mixture was then made up to 50 cc. The 7.5 percent solution was prepared in the same way except that 3.70 g of sodium cymenesulphonate and 0.05 g of cymenesulphonic acid were used to give the correct concentration and a pH value of 4.6.

The refractive index of the solution was determined, and 5 g of casein was added. The flask was stoppered, placed in an incubator held at the required temperature, and shaken at intervals.

Equilibrium was ascertained by noting or observing the constancy of the maximum of the refractive index over a period of, usually, from 2 to 4 days. Ordinarily, all salts required an average of about 10 or 11 days to attain equilibrium at 15° C., 7 or 8 days at 30°, and 4 or 5 days at 45°.

Percentages of casein were determined by the method of precipitation with 10 percent acetic acid solution, and weighing the casein directly as described above. Some of the early results were also checked by the Van Slyke gasometric micro-Kjeldahl method (*34*, *35*) for determining nitrogen.[4] Results were usually reproducible within narrow limits, although in some cases they were not as close as could be obtained with crystalloidal materials. Proteins, because of their indefiniteness and the huge size of their molecules, never give as exact results as do simpler inorganic and organic compounds.

Final solubility values for all salts (except as otherwise noted) were determined at 15°, 30°, and 45° C. These were considered the most practical temperatures to employ, because of the limiting effect of viscosity. Solubility values are reported as grams of casein dissolved in 100 g of solvent. The solubility values are listed in table 1.

TABLE 1.—*Solubility of casein in solutions of salts of organic acids having a pH value of 4.6*

Kind and concentration of the salt solution	Quantity of casein dissolved in 100 g of the solvent at—		
	15° C.	30° C.	45° C.
Sodium cymenesulphonate:	*Grams*	*Grams*	*Grams*
5 percent	3.0480	5.9390	9.6753
7.5 percent	5.2999	8.0197	
Potassium thiocyanate:			
2.5 percent	1.7850	2.5089	3.4201
5.0 percent	3.5490		
Sodium benzenesulphonate:			
5.0 percent	.9778	1.9331	3.0486
10.0 percent	2.2070	2.7450	4.8202

SOLUBILITY OF CASEIN IN POTASSIUM THIOCYANATE SOLUTIONS

The potassium thiocyanate used was a chemically pure analyzed salt. Potassium thiocyanate solution was found to be an excellent solvent for casein. Experimentation established that a 2.5-percent solution of potassium thiocyanate was best adapted to the method of sampling and to the temperatures employed. A 5-percent solution could be used at 15° C., but at 30° and 45° the solution became too dense.

In general, the 2.5-percent solution of potassium thiocyanate was prepared as follows: 1.25 g of KCNS was weighed out, 2 cc of phenol solution was added, and the solution was made up to the mark in a 50-cc volumetric flask. Rather surprisingly, the solution gave at this concentration a pH value close to 4.6. The 5-percent solution was made up somewhat similarly, except that 2.5 g of KCNS was used, and 8 drops of 0.1 N KOH (containing 0.0022 g of alkali) were necessary at this concentration to bring the solution to pH 4.6.

Five grams of casein was added for each determination. Much the same procedure was followed as was used with sodium cymenesulphonate solutions, although in some cases the time required for the solution to reach equilibrium was a day or two longer. The results are shown in table 1.

[4] The authors are indebted to P. D. Watson, of the Division of Dairy Research Laboratories, for making some of the analyses for phosphorus and nitrogen.

SOLUBILITY OF CASEIN IN SODIUM BENZENESULPHONATE SOLUTIONS

The sodium benzenesulphonate used was a commercial product of high purity. It was found that 5- and 10-percent solutions were the most practical to use.

The 5-percent solution was prepared by dissolving 2.5 g of sodium benzenesulphonate in water and adding 2 cc of phenol solution (containing 0.10 g). About 0.25 cc of 0.2 N NaOH solution (containing 0.002 g) was required to give a pH value of 4.6. The volume was made up to 50 cc, and 5 g of casein was added as before. The 10-percent solution was prepared in the same way, except that 5 g of salt was used and about 0.5 cc of 0.2 N NaOH solution (containing 0.004 g) was added to give the required pH value. Equilibrium was attained normally at the customary three temperatures. These results are also given in table 1.

On plotting the solubility values for casein in the 5-percent solution it was noted that the solubility curve was practically a straight line. For the 10-percent solution, there was a slight curve in the line. It was thought advisable to check again very carefully the points on the curve; the values obtained by the recheck showed that the points were correct.

FRACTIONATION OF ACID-PRECIPITATED CASEIN

EARLIER INVESTIGATIONS

Several investigators have believed that acid casein as ordinarily precipitated from milk is not a homogeneous compound but a mixture of closely related proteins or fractions.

According to Linderstrøm-Lang (*20*), in 1885, Danilewsky and Rodenhausen extracted a substance from casein by boiling it with a 50-percent alcoholic solution and then by means of sulphur determinations they sought to prove a difference between the extract and the residue. In 1918, Osborne and Wakeman (*26*) treated casein with a 92-percent alcoholic solution and obtained a fraction which differed considerably from casein in phosphorus content as well as in other particulars.

In 1925, Linderstrøm-Lang and Kodama (*21*) in determining the solubility of casein in hydrochloric acid in the presence of sodium chloride, observed that the solubility increased with the amount of casein added, which suggested that the protein was not a homogeneous material. They apparently proved by the phase rule that there were present more than the four components, i. e., casein, water, sodium chloride, and hydrochloric acid. They succeeded in separating casein into two fractions. The possibility of decomposition by the strong acid at the temperature used was ruled out by formol titrations and by constant solubility values day after day.

Svedberg, Carpenter, and Carpenter (*32, 33*) found by the Svedberg sedimentation-velocity method and the sedimentation-equilibrium method that Hammarsten casein appeared to contain several proteins of different molecular weight. By extracting casein with alcoholic HCl, they obtained a definite fraction which gave a molecular weight of $375{,}256 \pm 11{,}000$. Carpenter and Hucker (*5*) also found, by treating casein with acid alcohol and by fractionating it with potassium oxalate, that apparently three proteins having molecular weights of

98,000, 188,000, and 375,000 could be obtained from casein. According to Carpenter, hydrolysis of casein takes place very slowly.

In 1927–29, Linderstrøm-Lang (*20*) fractionated casein by means of alcoholic HCl at 60° to 70° C. He claimed that phosphorus-nitrogen ratios were excellent bases of differentiation between fractions. For example, in one fraction he found a P/N ratio of 0.0337 and in another fraction a P/N ratio of 0.0490. He postulated that either the casein is very unstable and easily breaks down into its components or else it consists of different molecules which may be slowly separated from each other. He favored the latter view. He found the yields of fractions were not quantitative; the correct proportions were not known. His results were reproducible, although he stated that—

> naturally such an experiment cannot be reproduced with the same degree of accuracy as solubility determinations with crystalline substances. One cannot expect that a fractionation of this kind will be able to give quite accurately identical results each time it is conducted. There is the possibility that milk from different cows can vary in its composition.

Linderstrøm-Lang concluded that casein is a mixture of colloidal molecules that form a coprecipitation system, and thus to a certain extent seem to act as a unit. He thought a study of fractionation should contribute much to the knowledge of the properties and reactions of casein.

Sørensen (*30*) made measurements of the solubility of proteins in salt solutions and found that solubility would increase with added amounts of proteins, thus indicating the presence of more than one solid phase. He assumed, however, that the proteins were not primarily mixtures of fractions, but "a complex combination of several components held together by residual valences." He presumed this compound to be easily dissociated into its components by the weak solvents employed, but he did not state whether the dissociation increased with time. If it did, this would be a powerful argument in favor of the dissociation theory.

Cherbuliez and Schneider (*6*) in 1932 concluded that casein was a mixture of inconstant composition. They used ammonium chloride as the fractionating agent. They stated that casein is very sensitive to the action of water when the pH value of the solution is above 7, but that it is probably perfectly stable in weakly acid solutions as at pH 4.6. These workers found that the components possessed different solubilities in 70-percent pyridine solutions and varied in their rennet-coagulation characteristics.

Berggren (*3*) separated casein by HCl and lactic acid and a dialysis procedure. She found P/N ratios of 0.0522 and 0.0558 and 0.0444 and 0.0215.

In 1934, Gróh (*15*) fractionally precipitated casein from its 40-percent urea solution and its solution in molten phenol by adding absolute ethanol, and from a 70-percent alcoholic ammonium hydroxide solution by adding dilute HCl. The initial fractions were further divided into subfractions. No evidences of hydrolysis were found.

Giza (*11*) separated casein by Linderstrøm-Lang's method, and found that fractions differed in phosphorus and in amino acid content as shown by spectrophotometric analysis. He also observed variations in the buffer capacities.

Jirgensons (*17*, *18*) fractionated Hammarsten casein, and precipitated the components by various concentrations of methanol, ethanol, and propanol. Results were plotted. Apparently a uniform protein will give a curve with a single maximum and minimum point, whereas a nonuniform protein ordinarily does not.

In 1935, Carpenter (*4*) postulated that the reversible dissociation of casein would take place at neutrality by dilution with a large volume of buffer solution.

Supplee (*2*, *p. 52*) brought about fractionation of casein by successive sedimentation and washing of fine casein. He found one fraction in particular to be very high in phosphorus.

In 1936, Pedersen (*27*) concluded that casein would dissociate during sedimentation and produce smaller molecules.

EXPERIMENTAL PROCEDURE AND EVIDENCES OF FRACTIONATION

As explained before, the possibility of selective solubility, or fractionation effect, was considered in the course of the solubility determinations. Primary evidence of fractionation was afforded if the refractometer showed an increased index on the addition of further amounts of casein after equilibrium had been attained with the quantity already present. This result, if obtained, was checked by further tests such as phosphorus and nitrogen determinations, formaldehyde absorptions, and solubilities in pyridine solution.

Some hint of fractionation was noticed in an early experiment with sodium cymenesulphonate solution. Seven and five-tenths grams of casein was the starting quantity, and an equilibrium refractive index of 1.3972–3 was first reached. On the addition of a second increment of 5 g of casein, the index jumped to 1.4030 in 1½ hours, indicating the extraction of material from the added casein. As is well known, if the solid phase is homogeneous, the solubility value at equilibrium will not be enhanced by the addition of further amounts of the solid phase. On the other hand, if the solid phase is heterogeneous, the quantities dissolved by the solvent may increase in proportion to the further increments of the material added, provided the solubility of the most soluble fraction has not been exceeded.

The same phenomenon (marked increase of the refractive index on addition of further quantities of casein) was noticed both with a 7.5-percent and a 5-percent sodium cymenesulphonate solution at 45° C., and a 2.5-percent potassium thiocyanate solution at 30°. In the latter case, fractionation was not confirmed by the P/N ratio determinations.

FRACTIONATION WITH 5-PERCENT SODIUM BENZENESULPHONATE SOLUTION

Grove City Casein

A 5-percent solution of sodium benzenesulphonate was examined for possible fractionating tendencies. A solution containing 2.5 g of the salt, 0.25 cc of 0.2 N NaOH solution (containing 0.002 g), and 2 cc of phenol solution was made up to a volume of 50 cc. This solution had a pH value of 4.6, and 45° C. was the temperature of the run. Two grams of Grove City casein was added at first. After 4 days the refractive index became stabilized. Two grams of casein was again added, and after 2 days the refractive index showed a second equi-

librium point, thus indicating the possibility of fractionation. One gram more of casein was added at this point, but shortly thereafter the thermostatic control of the incubator failed to function. The solution was then removed from the incubator, as it was thought that enough of the fractions were present to show tests. The solution was filtered through canvas, the residue washed free of sodium benzenesulphonate, as shown by the silver nitrate test, and finally washed with ethanol and ether. The substance dissolved in the filtrate was precipitated with 10-percent acetic acid, filtered, and washed in the same manner. The residue and the soluble material were dried in a vacuum desiccator over calcium chloride. In later experiments, the residue and the soluble material were more effectively dehydrated by washing with acetone and ether, and then drying in the oven at 75° to 80°.

Cherbuliez and Schneider (*6*) recommended a 70-percent aqueous pyridine solution as a reagent for distinguishing, on a basis of varying solubilities, different casein fractions. They also made use of the rennet-coagulation test to show the same distinction. These tests were applied to the residue and the soluble material obtained in this experiment. One-tenth gram of the residue fraction was added to 20 cc of a 70-percent aqueous pyridine solution. After 24 hours, the residue was only partly dissolved. The fraction obtained from the filtrate was similarly tested and it was found to be completely soluble in only a few hours. The rennet test was also applied to each fraction. To 0.1 g of each fraction was added 0.12 g of calcium hydroxide, 3.2 cc of water, and 0.16 cc of rennet solution. No coagulation was observed in the solution of either fraction.

TABLE 2.—*Results of fractionating different kinds of casein with different agents* [1]

Kind of casein and fraction	Phosphorus	Nitrogen	P/N ratio	Formaldehyde absorbed	Solubility in pyridine
SODIUM BENZENESULPHONATE (5 PERCENT)					
	Percent	*Percent*		*Milligrams*	
Grove City casein:					
Insoluble residue	0.849	15.67	0.0542		Part.
Fraction from filtrate	2.22	15.61	.1422		Entire.
Whittier casein:					
Insoluble residue	.865	15.67	.0552		
Fraction from filtrate	.78	15.67	.0500		
SODIUM BENZENESULPHONATE (10 PERCENT)					
Whittier casein:					
Insoluble residue	0.844	15.62	0.0538	2.4	Slight.
Fraction from filtrate	.912	15.68	.0582	1.2	Entire.
Hammarsten casein:					
Insoluble residue	.786	15.67	.0502	4.7	Slight.
Fraction from filtrate	1.87	15.65	.1197	3.9	Entire.
SODIUM CYMENESULPHONATE (5 PERCENT)					
Hammarsten casein:					
Insoluble residue	0.785	15.67	0.0502	4.3	Slight.
Fraction from filtrate	1.21	15.62	.0774	1.7	Part.
Whittier casein:					
Insoluble residue	.907	15.64	.0576	5.4	Part.
Fraction from filtrate	.839	15.65	.0536	2.8	Slight.

[1] Results listed were calculated on a dry, ashless basis.

Phosphorus and nitrogen analyses were made on the two fractions. The results are shown in table 2.

The phosphorus-nitrogen ratios thus offer strong evidence that the sodium benzenesulphonate solution has fractionated the casein into components of widely differing phosphorus percentages. This evidence was confirmed refractometrically and by pyridine solubility tests.

Whittier Casein

The preceding experiment was repeated in the same way except that 5 g of Whittier casein was used. After completion of the run, the residue was washed with water, acetone, and ether. Acetone was substituted for the ethanol used in the preceding experiment because of the possibility that some alcohol-soluble component might be extracted, and also because of the statement by Cherbuliez and Schneider (*6*) that acetone helps to produce a powdery product that does not stick to the filter. The fraction in the filtrate was precipitated with 10-percent acetic acid at 35° to 40° C. The precipitate at this temperature is granular and easily filtered. At lower temperatures, a fine product is produced which clogs the filter. At higher temperatures a gummy substance is formed which cannot be washed effectively. The fraction from the filtrate was washed in the same way as the residue, and then both fractions were dried overnight in the oven at 75° to 78°.

Phosphorus and nitrogen determinations were made on these fractions. Nitrogen values were obtained by the Kjeldahl-Gunning method. Blanks were run to determine the purity of the reagents. The factor used for converting nitrogen to protein was 6.38. Phosphorus was estimated by a method for analyzing phosphorus in casein previously adapted by the authors from the methods of Meigs, Blatherwick, and Cary (*25*) and Fales (*10, pp. 213–228*).

The analytical results of this second fractionation are shown in table 2.

Some fractionation is thus indicated, although not as much as when Grove City casein was used, even though this experiment was run for a shorter time.

Different caseins vary in their proportions of fractions, according to the writers' observations and also those of Linderstrøm-Lang (*20*).

Fractionation with 10-Percent Sodium Benzenesulphonate Solution

Whittier Casein

Twenty-five grams of sodium benzenesulphonate, 2.5 cc of 0.2 N NaOH solution, and 0.5 g of phenol were made up to 250 cc of solution. This had a pH value of 4.6. Twenty-five grams of Whittier casein was added, the solution was placed in an incubator held at 45° C., and the mixture was shaken at intervals. After 6 days, equilibrium was proved by the constancy of the refractive index at 1.3602. The residue was filtered with suction and through canvas on a Büchner funnel. Because of the larger quantities of casein used, filtration by this method was extremely slow, requiring fully 3 weeks. The fraction in the filtrate was precipitated with 10-percent acetic acid solution. Both fractions were washed with water, acetone, and ether, and dried in the oven at 75° to 80°.

The residue and the fraction precipitated from the filtrate were analyzed for nitrogen by the Kjeldahl-Gunning method, and for phosphorus by the method described on page 135. The values are presented in table 2.

Some fractionation was again obtained. However, it is evident that partial qualitative but not complete or quantitative fractionation may be achieved by the use of the solvents employed. As shown later, fractionation was more definitely evidenced by the formaldehyde absorption and pyridine solubility tests.

Formaldehyde absorption.—Additional tests for fractionation were sought. It was postulated that the two fractions might vary in their affinity or absorbing capacity for formaldehyde. This would imply a difference in the number of free amino groups in each fraction, since formaldehyde is generally considered to react with the casein molecule as follows:

$$RNH_2+HCHO\rightarrow RN:CH_2+H_2O.$$

A test of the applicability of this reaction was carried out by exposing the fractions in a desiccator to the vapors of a 37-percent formalin solution. The fractions increased in weight at different rates, but since water vapor was absorbed from the formalin solution along with formaldehyde, a second variable was introduced which affected the reliability of the results.

Later, experiments were run in which the formalin solution was replaced by solid paraformaldehyde. This eliminated the water variable. The paraformaldehyde gradually gave off formaldehyde vapor which was absorbed.

The fractions were screened in order to aid in exposing equal surface areas. Those portions that passed through a No. 40 screen and were retained on a No. 60 screen, were taken for the absorption tests. Two samples of each fraction were weighed into weighing bottles and run simultaneously in the same desiccator. This was kept in the 30° C. incubator. At intervals, the dishes were removed from the desiccator, covered, and the time of covering was taken. The bottles were weighed after being allowed to come to room temperature. After each weighing, the solids in the dishes were mixed by gently shifting them back and forth in order to expose new surfaces to the action of formaldehyde. The absorption proceeded very rapidly for the first day or two, after which it advanced more slowly. Intermediate values did not always check closely, probably because of changes in exposed surfaces; but as the run proceeded, better checks were obtained until final values, after 13 days, were in close agreement. The average of checks for these final values were: For the residue, 2.4 mg; for the fraction from the filtrate, 1.2 mg. Thus the first fraction absorbs twice as much formaldehyde as the second, strongly indicating that there are more free amino groups present in the residue than in the filtrate fraction.[5] This test furnishes an additional proof of fractionation.

Pyridine solubility.—Two-hundredths of a gram each of the residue and the fraction from the filtrate were placed in stoppered test tubes, each tube containing 4 cc of 70-percent aqueous pyridine solution.

[5] It was first planned to use the formaldehyde-absorption test as a dynamic one, but intermediate rates of reaction did not check because of differences in particle size. Accordingly, only final weights of formaldehyde absorbed by the fractions were recorded.

The tubes were warmed to 40° to 45° C. for 1 hour, and then held at room temperature for 24 hours, during which time they were shaken frequently. The residue was only slightly soluble in the pyridine solution. The fraction from the filtrate was entirely soluble.

Hammarsten Casein

The preceding experiment was repeated with a different sample of casein—i. e., Hammarsten—to observe how caseins from different sources compared, and to check further the fractionating characteristics of sodium benzenesulphonate. The same kinds and quantities of materials were employed except 25 g of Hammarsten casein was used instead of Whittier casein. The conditions of the determination at 45° C. were practically the same.

Because of the extremely slow filtration resulting from the use of a Büchner funnel and vaccum filtration, a pressure filter was constructed and employed thereafter in this and the subsequent larger-scale fractionations. The pressure filter aided greatly in speeding up the time of filtration, only 9 days being required to complete this fractionation as compared to 3 weeks for the preceding one. The fractions obtained were treated by the usual procedure.

The results of the phosphorus and nitrogen analyses are shown in table 2.

Extensive fractionation is shown by the difference in the P/N ratios. Hammarsten casein is obviously fractionated to a greater degree than the Whittier casein by the sodium benzenesulphonate solution.

Not enough material was available from this particular fractionation for additional tests. Accordingly, a second experiment was carried out to obtain additional quantities of the components.

Considerably larger quantities were used this time; i. e., 50 g of sodium benzenesulphonate, 5 cc of 0.2 N NaOH, and 1.0 g of phenol were made up to 500 cc, and 50 g of Hammarsten casein was used. The action of the solvent on the casein was continued for 6 days.

The filtration of the larger quantity of residue presented much difficulty, even with a pressure filter. Parts of the mixture were finally removed and filtered by using two Büchner funnels to speed up the process. This involved some loss of material. The filtration required over 3 weeks. The fractions obtained were treated as before. When the yields were weighed, the residue was 29.40 g and the fraction from the filtrate was 1.50 g.

Formaldehyde absorption.—Screened 20-mg samples of these fractions were placed in a desiccator, over solid paraformaldehyde, and allowed to absorb formaldehyde vapors. Final values were recorded after 12 days, although absorption was still proceeding at a very slow rate. Ordinarily, 12 or 13 days is sufficient time to attain good checks and establish differences between the fractions. The final values were: For the residue, 4.7 mg; for the fraction from the filtrate, 3.9 mg.

Pyridine solubility.—Two-hundredths of a gram of each fraction was taken for the test and the same method was applied as outlined previously. The residue was only slightly soluble in 4 cc of 70-percent pyridine solution, whereas the fraction from the filtrate was wholly soluble.

The pyridine solubility test probably shows differences in the acidic groups of the fractions, whereas the formaldehyde absorption test very likely indicates differences in the free amino groups.

FRACTIONATION WITH 5-PERCENT SOLUTIONS OF SODIUM CYMENESULPHONATE

HAMMARSTEN CASEIN

Recrystallized sodium cymenesulphonate was used for this experiment. Two and forty-seven hundredths grams of salt was weighed out, cymenesulphonic acid solution equivalent to 0.03 g was added (to give the usual pH value), 2 cc of phenol was added and the solution was made up to 50 cc. Small portions of Whittier casein were poured in from time to time and the subsequent establishment of successive constant refractometric readings furnished some indication of fractionation.

The mixture was filtered, and the fractions were prepared in the regular way.

The pyridine and rennet tests were applied to these components as in preceding experiments. The fraction precipitated from the filtrate was largely dissolved in the 70-percent pyridine solution; the residue was not so soluble. The rennet test was negative in both cases. There was not enough material left for phosphorus and nitrogen determinations. Accordingly, another fractionation was attempted on a larger scale.

The solution was prepared in this way: 12.35 g of recrystallized sodium cymenesulphonate was mixed with 0.9 cc of cymenesulphonic acid solution (containing 0.1376 g per cc), 0.5 g of phenol was added, and the solution was diluted to 250 cc. It was now at the usual pH value. A 25-g sample of Hammarsten casein was used for the experiment.

Fractionation was complete in 6 days, and the solution was placed in the pressure filter. Filtration proceeded rapidly, and was finished in 3 days. The fraction from the filtrate was precipitated and both fractions were washed and dried in the customary manner. Lead nitrate solution was used to test for completeness of washing. In this test any residual cymenesulphonate will come down in white flakes as the lead salt.

Nitrogen and phosphorus values are recorded in table 2.

These values help to prove that 5-percent sodium cymenesulphonate solution is an effective fractionating agent since the P/N ratios show a wide divergence.

Formaldehyde absorption.—The residue showed a total increase of 4.3 mg, and the fraction from the filtrate, 1.7 mg. Strong confirmatory evidence of fractionation is hereby afforded.

Pyridine solubility.—The difference in solubility was not as marked in these fractions as in the fractions obtained by sodium benzenesulphonate solution. The fraction from the filtrate was evidently more soluble in the pyridine solution than the residue.

WHITTIER CASEIN

The experiment with Whittier casein was on a larger scale than the preceding ones. Forty-nine and four-tenths grams of recrystallized sodium cymenesulphonate solution was weighed out, 3.6 cc of cymenesulphonic acid solution (containing 0.1376 g per cc) was poured in to establish the correct acidity, 2 g of phenol was added, and the total mixture was diluted to 1,000 cc. One hundred grams of Whittier casein was used and the fractionation was carried on for 7 days at 45° C.

Considerable difficulty was experienced with the pressure filter in attempting to handle this large quantity of material, and some was later transferred in successive increments to a Büchner funnel. Some material was lost because of this and other difficulties. Precipitation, washing, and drying were carried out as with the Hammarsten casein. Yields were: Residue, 16.20 g; fraction from the filtrate, 36.90 g.

Phosphorus and nitrogen results are listed in table 2.

Fractionation is again indicated, but in a different manner from that in which it took place when Hammarsten casein was used.

Formaldehyde absorption.—Final weights were taken in 12 days, and were: Residue, 5.4 mg; fraction from the filtrate, 2.8 mg.

Pyridine solubility.—In this particular case it was very difficult to distinguish between fractions, although the residue appeared to be slightly more soluble than the fraction from the filtrate.

DISCUSSION AND SUMMARY

The question of possible hydrolysis of the protein is often raised in connection with fractionation work. However, under the experimental conditions used in this research, the chances of any hydrolytic decomposition are nearly negligible. No evidence of such decomposition (provided a suitable preservative was used) was obtained in the course of the experimentation. The attainment of equilibrium, as shown by constancy of the refractive index, was not dependent on time as would have been the case if hydrolysis were proceeding. The fractions in the various filtrates were always coagulable by acids. Even assuming the presence of any hydrolytic products, most of them would have been leached out by the washing liquids when the fractions were washed, and so would not affect the phosphorus-nitrogen ratios or other tests. Furthermore, the experiments were run at the isoelectric point of casein, a pH value of 4.6, the point at which there is the greatest stability and the least chance of decomposition.

The work of other investigators revealed no evidence of hydrolysis during fractionation even when much stronger reagents were used. (See Linderstrøm-Lang (*20*) and Kondo (*19*)). Soff (*29*) found no indications of hydrolysis even after long storage of casein that was dissolved in the sulphonate solutions.

SOLUBILITY OF CASEIN

From the solubility values tabulated in table 1, it will be seen (on the basis of the solubility determined at each of the three temperatures) that in almost all cases solubility increased with the temperature as a straight-line function. With a 10-percent solution of sodium benzenesulphonate, a slight curve was obtained. In the case of a concentrated solution like this, however, some minor deviation from an ideal solubility curve is not unexpected. Increased solvate formation with the sulphonate may be a factor. It will be observed that the determinations generally intercheck, i. e., they rise in a straight line and increase in definite increments between temperatures.

The solubility of casein (on the basis of 5 g to 50 cc of solution at a pH value of 4.6) in a 5-percent aqueous solution of sodium cymenesulphonate at a constant temperature of 15°, 30°, and 45° C. has been

determined. For 7.50-percent solutions, values at 15° and 30° only could be obtained. The solubilities shown by these determinations were a straight-line function of the temperature.

In the same way, the solubility of casein in a 2.5-percent solution of potassium thiocyanate at 15°, 30°, and 45° C. has been established. For a 5-percent solution of potassium thiocyanate, only the solubility at 15° C. could be determined. For the 2.5-percent solution, the solubility increased with the temperature, and was plotted as a straight line.

The solubility of casein has also been determined in 5-percent and 10-percent sodium benzenesulphonate solutions at 15°, 30°, and 45° C. For the 5-percent solution, the solubility increased in a straight line in the same manner as with the other solvents; but for the 10-percent solution, the solubility showed a slightly curved line, as previously explained.

Potassium thiocyanate solutions were found, for the first time, to be solvents for casein.

The use of the formation of lead cymenesulphonate and silver benzenesulphonate as tests for completeness of washing was developed.

FRACTIONATION OF CASEIN

It has been shown for the first time that sodium benzenesulphonate and sodium cymenesulphonate solutions at a pH value of 4.6 will separate casein into at least two components. These components, or fractions, exhibited chemical differences in that their percentages of phosphorus varied; their formaldehyde-absorbing capacities differed (probably indicating unlike numbers of free amino groups); and their solubility values in pyridine solutions varied (evidently showing differences in acidic constituents).

Potassium thiocyanate solution was not a fractionating agent.

Salts of nearly neutral action, such as these sulphonates, should be of great general value for fractionation purposes since the possibility of hydrolyzing proteins is reduced to a minimum. Emphatic criticism of the use of strong acids and alkalies as fractionating reagents has been voiced by some protein chemists because of the likelihood that the molecules may be broken down by such drastic treatment. The use of the nearly neutral sulphonates should obviate any such contingency.

It appears from an inspection of table 2 that qualitative fractionation has been achieved with these agents, but not quantitative fractionation. Strong evidences of fractionation were supplied in the experiments with the Grove City casein and the Hammarsten casein, but when Whittier casein was used the indications of fractionation were not so pronounced. This may have been due to differences in the caseins themselves, or in the methods of preparation. Svedberg, et al. (*32, 33*) stated that the molecular weight and homogeneity of casein depended on the way in which it had been prepared. There is a possibility that partial pre-fractionation took place during the preparation of the Whittier casein as it was first washed with dilute HCl. (See Linderstrøm-Lang's procedure, p. 132.) The casein was also washed with water in tall cylinders for a considerable period of time, and some of the high-phosphorus fraction might have been separated by sedimentation. (See Supplee's method, p. 133.) Linderstrøm-

Lang (*20*) commented on the diverse ratios of the fractions in casein from different sources. Possibly, too, Sørensen's theory of the reversible dissociation of casein (*30*) might explain the lack of fractionation to uniform definite limits in all cases, since the degree of dissociation might vary with many factors involving the casein and reagent used. It will be observed that, when Whittier casein was fractionated with a 5-percent sodium benzenesulphonate solution, the P/N ratios showed an opposite trend from those obtained when fractionation was with a 10-percent solution. This may be due to differing selective solubilities of various concentrations of the salt; or if Sørensen's theory is correct, to the differing dissociating characteristics of the 5-percent and 10-percent solutions.

A new method of testing for differences in fractions has been developed which proves that the amounts of formaldehyde absorbed from paraformaldehyde by the components vary considerably. Results evidently indicate that the fractions are unlike in free amino group contents.

The pyridine solubility test has been employed as a third confirmatory method to further establish the fact of component separation. Differences in the solubility of the fractions are probably due to variations in the acidic groups present.

The refractive indices of the solutions also furnished indications of fractionation; the indices increased beyond previous equilibrium points on the addition of successive increments of casein.

The theory that casein is a mixture or a composite of loosely bound aggregates, rather than a compound, receives emphatic substantiation since a fractionation into constituents has been effected by the nearly neutral, nonhydrolyzing salts of organic acids. The fact that fractionation equilibrium was not dependent on time (as shown by the refractometer) lends evidence to Linderstrøm-Lang's casein-mixture theory rather than to Sørensen's casein-aggregate hypothesis. A further investigation of the fractions obtained should clarify even more the composition and structure of casein.

LITERATURE CITED

(1) Agthe, Carl Alexander.
1932. verfahren zur herstellung von neutralen oder schwach sauren wasch- und emulgierungsmitteln. German Patent 547,895. Ausgegeben Apr. 1, 1932.

(2) Associates of Rogers, Lore A.
1935. fundamentals of dairy science. Ed. 2, 616 pp., illus. New York.

(3) Berggren, Ruth E. L.
1932. the phosphorus content of casein. preliminary paper. Jour. Biol. Chem. 95: 451–460, illus.

(4) Carpenter, D. C.
1935. the optical rotation and dissociation of casein. Jour. Amer. Chem. Soc. 57: 129–131, illus.

(5) ——— and Hucker, G. J.
1930. serologic studies on the proteins found in casein. Jour. Infect. Diseases 47: 435–442.

(6) Cherbuliez, Emile, and Schneider, Margarethe Lily.
1932. recherches sur la caséine. i. la caséine n'est pas un corps homogène. étude de son fractionnement par le chlorure d'ammonium. Helvetica Chim. Acta 15: 597–609, illus.

(7) COHN, EDWIN JOSEPH.
1922. STUDIES IN THE PHYSICAL CHEMISTRY OF THE PROTEINS. I. THE SOLUBILITY OF CERTAIN PROTEINS AT THEIR ISOELECTRIC POINTS. Jour. Gen. Physiol. 4: 697–722, illus.
(8) COHN, EDWIN J., and HENDRY, JESSIE L.
1923. STUDIES IN THE PHYSICAL CHEMISTRY OF THE PROTEINS. II. THE RELATION BETWEEN THE SOLUBILITY OF CASEIN AND ITS CAPACITY TO COMBINE WITH BASE. THE SOLUBILITY OF CASEIN IN SYSTEMS CONTAINING THE PROTEIN AND SODIUM HYDROXIDE. Jour. Gen. Physiol. 5: 521–554, illus.
(9) EULER, H. v., and BUCHT, BIRGER.
1923. ZUR KENNTNIS DER SORPTIONSGLEICHGEWICHTE. Ztschr. Anorgan. u. Allg. Chem. 126: 269–277, illus.
(10) FALES, HAROLD A.
1925. INORGANIC QUANTITATIVE ANALYSIS. 493 pp., illus. New York.
(11) GIZA, T.
1934. RESEARCHES ON CASEIN. Bull. Internatl. Acad. Polonaise Sci. et Lettres, Cl. Sci. Math. et Nat. (A) (10): 421–447, illus.
(12) GOLDSMITH, BYRON B.
1907. CASEIN COMPOUND. U. S. Patent 840,931. U. S. Patent Office Off. Gaz. 126: 626–627.
(13) ———
1907. IMPROVEMENTS IN AND CONNECTED WITH CASEIN COMPOUNDS OR ARTICLES. Brit. Patent 14,098. A. D. 1907. Applied for June 18, 1907; accepted July 20, 1908.
(14) ———
1909. PROCESS OF MAKING CASEIN AND LIKE COMPOUNDS. Brit. Patent 412, A. D. 1909. Applied for Jan. 7, 1909; accepted Jan. 7, 1910.
(15) GRÓH, J.
1934. ÜBER DIE FRAKTIONIERUNG DES CASEINS. Hoppe-Seyler's Ztschr. Physiol. Chem. 226: 32–44.
(16) ISGARISCHEW, N., and POMERANZEWA, A.
1926. DIE QUELLUNG IN GEGENWART ORGANISCHER SÄUREN IM ZUSAMMENHANG MIT DEREN STRUKTUR. Kolloid Ztschr. 38: 235–238.
(17) JIRGENSONS, BR.
1934. ÜBER DIE BESTIMMUNG DER EINHEITLICHKEIT VON PROTEINEN DURCH FLOCKUNGSVERSUCHE. Biochem. Ztschr. 268: [406]–413, illus.
(18) ———
1934. UNTERSUCHUNGEN ÜBER DIE FRAKTIONEN DES CASEINS. Biochem. Ztschr. 268: [414]–421, illus.
(19) KONDO, KINSUKE.
1925. STUDIES ON CASEIN. Compt. Rend. Trav. Lab. Carlsberg 15, No. 8, 39 pp., illus.
(20) LINDERSTRØM-LANG, K.
1929. STUDIES ON CASEIN. III. ON THE FRACTIONATION OF CASEIN. Compt. Rend. Trav. Lab. Carlsberg 17, No. 9, 116 pp., illus.
(21) ——— and KODAMA, SAKUJI.
1925. STUDIES ON CASEIN. I. ON THE SOLUBILITY OF CASEIN IN HYDROCHLORIC ACID. Compt. Rend. Trav. Lab. Carlsberg 16. No. 1, 62 pp., illus.
(22) LOISELEUR, J.
1932. SUR QUELQUES PROPRIÉTÉS DES PROTÉIDES EN SOLUTION ORGANIQUE. Bull. Soc. Chim. Biol. 14: [1088]–1100.
(23) ——— and VELLUZ, L.
1932. PRÉPARATION ET PROPRIÉTÉS DES MEMBRANES PROTÉO-CELLULOSIQUES. Bull. Soc. Chim. Biol. 14: [1210]–1217.
(24) LOISLEUR, JEAN and VELLUZ, LÉON.
1931. SUR L'ASSOCIATION DE CONSTITUANTS BIOCHEMIQUES ET DE CERTAINS ÉTHERS CELLULOSIQUES. Compt. Rend. Acad. Sci. [Paris] 192: 159–161.
(25) MEIGS, EDWARD B., BLATHERWICK, N. R., and CARY, C. A.
1919. CONTRIBUTIONS TO THE PHYSIOLOGY OF PHOSPHORUS AND CALCIUM METABOLISM AS RELATED TO MILK SECRETION. Jour. Biol. Chem. 37: 1–75, illus.

(26) OSBORNE, THOMAS B., and WAKEMAN, ALFRED J.
1918. SOME NEW CONSTITUENTS OF MILK. THIRD PAPER. A NEW PROTEIN, SOLUBLE IN ALCOHOL. Jour. Biol. Chem. 33: 243–251.
(27) PEDERSEN, KAI OLUF.
1936. ULTRACENTRIFUGAL AND ELECTROPHORETIC STUDIES ON THE MILK PROTEINS. I. INTRODUCTION AND PRELIMINARY RESULTS WITH FRACTIONS FROM SKIM MILK. Biochem. Jour. 30: 948–960, illus.
(28) SCHWARZBERG, HANS.
1910. VERFAHREN ZUR HERSTELLUNG EINER HORNARTIGEN MASSE. German Patent 225,259, Patentiert Apr. 28, 1904; ausgegeben Sept. 26, 1910. German Patent 229,906, Zusatz zum Patente 225,259, Ausgegeben Jan. 7, 1911.
(29) SOFF, LEROY D.
1932. ELIMINATION OF PIGMENT-DRYING (IN THE PRODUCTION OF WATER PAINTS, OIL PAINTS, AND LACQUERS). 30 pp. New York City. (Diss., Columbia Univ.).
(30) SØRENSEN, S. P. L.
1930. THE CONSTITUTION OF SOLUBLE PROTEINS (REVERSIBLY DISSOCIABLE COMPONENT SYSTEMS). Compt. Rend. Trav. Lab. Carlsberg 18, No. 5, 124 pp., illus.
(31) SUTERMEISTER, EDWIN.
1927. CASEIN AND ITS INDUSTRIAL APPLICATIONS. 296 pp., illus. New York.
(32) SVEDBERG, THE.
1926. ÜBER DIE BESTIMMUNG VON MOLEKULARGEWICHTEN DURCH ZENTRIFUGIERUNG. Ztschr. Phys. Chem. 121: 65–77, illus.
(33) ——— CARPENTER, L. M., and CARPENTER, D. C.
1930. THE MOLECULAR WEIGHT OF CASEIN. Jour. Amer. Chem. Soc. 52: 241–252, illus.
(34) VAN SLYKE, DONALD D.
1927. GASTROMETRIC MICRO-KJELDAHL DETERMINATION OF NITROGEN. Jour. Biol. Chem. 71: 235–248.
(35) ——— and KUGEL, VICTOR H.
1933. IMPROVEMENTS IN MANOMETRIC MICRO-KJELDAHL AND BLOOD UREA METHODS. Jour. Biol. Chem. 102: 489–497.
(36) VON WEIMARN, P. P.
1927. CONVERSION OF FIBROIN, CHITIN, CASEIN, AND SIMILAR SUBSTANCES INTO THE ROPY-PLASTIC STATE AND COLLODIAL SOLUTION. Indus. and Engin. Chem. 19: 109–110, illus.
(37) ———
1928. COLLODIAL DISSOLUTION OF COMPOSITE (HIGH-MOLECULAR) COMPOUNDS BY MEANS OF SUBSTANCES WHICH POSSESS TRUE SOLUBILITY IN ABUNDANCE, AND WHICH ARE INCLINED TO A GREAT SOLVATION. Japan. Jour. Chem. 3: 71–87.

RELATION OF BITTERNESS TO THE TOXIC PRINCIPLE IN SWEETCLOVER[1]

By WILLIAM K. SMITH, *agent, Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and assistant professor of genetics and agronomy, Wisconsin Agricultural Experiment Station;* and R. A. BRINK, *professor of genetics, Wisconsin Agricultural Experiment Station*[2]

INTRODUCTION

During the last three decades, sweetclover in North America has risen from the status of a roadside weed to a place of importance as a forage crop. This rapid adoption as a cultivated crop has taken place in spite of several undesirable characteristics. One of the factors at present limiting its usefulness is its rather bitter taste. Although livestock, at first reluctant to eat it, become accustomed to the taste, the bitterness may be a disadvantage when sweetclover is used for pasture in mixtures with grasses and other clovers. Another limiting factor is that unless sweetclover hay is thoroughly cured, certain changes may take place during storage in the stack or mow which render the hay toxic when fed to livestock. The resulting disease has been referred to as "sweetclover disease." Some studies on the relation between the two undesirable characteristics—bitterness and toxicity—are described in this paper.

THE SWEETCLOVER DISEASE

In 1922 Schofield (*9*)[3] called attention to a disease in cattle characterized by low clotting power of the blood and the development of extensive hemorrhages which were usually fatal. He showed the causal relation of the feeding of spoiled sweetclover hay or silage. Since that date, numerous reports of the occurrence of the disease have appeared. A careful series of studies on the pathology and therapy in cattle was made by Roderick and Schalk (*8*) with observations on the malady in other livestock. Roderick (*7*), working on cattle, has shown that in this disease the low clotting power of the blood is the result of a deficiency of prothrombin—one of the constituents of the blood essential for clotting. This finding has recently been confirmed by Quick (*5*) on rabbits by the use of the technique of Quick, Stanley-Brown, and Bancroft (*6*) for prothrombin determination.

Cannon and Greenwood (*4*) observed in rabbits a lowering of the calcium content of the blood on a diet of well-cured sweetclover hay and suggested that hemorrhage in sweetclover disease may be linked

[1] Received for publication February 7, 1938; issued August 1938. Contribution from the Department of Genetics (Paper No. 225), Wisconsin Agricultural Experiment Station, in cooperation with the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. Cooperative investigations with the biochemistry research laboratory, department of agricultural chemistry, Wisconsin Agricultural Experiment Station.

[2] The writers are pleased to acknowledge the interest and support of Dr. E. A. Hollowell in these studies. They are indebted also to Dr. Karl Paul Link for helpful counsel. Dr. W. L. Roberts of the biochemistry research laboratory prepared the coumaric acid, melilotic acid, and melilotic acid lactone used in the experiments. In the preparation of the melilotic acid lactone the cooperation of Dr. Homer Adkins in the catalytic high-pressure hydrogenation of coumarin is gratefully acknowledged.

[3] Italic numbers in parentheses refer to Literature Cited, p. 153.

with the decline in serum calcium. In testing this point with cattle, Brown, Savage, and Robinson (*3*) found that variations in blood calcium and phosphorus were not significant either when the animals were fed well-cured sweetclover hay or when the clotting power of the blood was low following a feeding period on a known toxic sweetclover hay.

MATERIALS AND METHODS

In the present studies rabbits were used exclusively. The rabbit consumes a small amount of feed, and as was first noted by Schofield (*10*), gives a prompter response than cattle. The progress of the disease has been followed by recording the clotting time of small samples of blood taken at intervals during the feeding period. Approximately one-half cc of blood is allowed to drop from a freely bleeding incision in the marginal vein of the ear into a 50-mm watch glass. Another watch glass of the same size is inverted over the first and the time required for clot formation is determined by tilting the watch glasses at 30-second intervals at a laboratory temperature of 23° to 24° C. In a normal rabbit a firm clot forms in 5 to 7 minutes. Young rabbits, 8 weeks old, are much more susceptible than mature animals, but among rabbits of the same age a marked difference in reaction to the toxic principle has been observed.[4] Some show a prolonged clotting time with a small amount of toxic hay, while others show no symptoms even on an exclusive diet of toxic hay over a prolonged period. Further, as Roderick (*7*) found to be the case with cattle, there is no indication in rabbits of an acquired resistance to the toxic principle. One can induce the symptoms of the disease in a susceptible rabbit, allow it to recover on a nontoxic diet, and in subsequent feeding of the same amount of toxic hay the reaction will be very similar, allowance being made for advancing age. For comparisons of the relative toxicity of spoiled hays, a group of rabbits is subjected to a preliminary feeding test on a known toxic hay in amounts proportional to the body weight (45 g per 1,000 g of body weight daily) and those showing symptoms promptly in the blood clotting test are transferred to nontoxic feed in an early stage of the disease. After an interval of several days, they may be used in comparative trials.

Toxic hay can be prepared by drying to 40 or 50 percent moisture freshly cut common sweetclover, stacking it, and covering the piles as described below for individual tests. In the experiments of Roderick and Schalk (*8*), the hay was allowed to remain in small piles for 60 days or more. The writers have found, however, that the hay may become toxic in a much shorter period. In one test, 290 pounds (green weight) of Hubam, an annual form of *Melilotus alba* Desr., cut when in midflower, was dried in the sun to 49 percent moisture, put in a pile in a basement laboratory, and covered to a depth of about 6 inches with slightly wilted third-cutting alfalfa. After 8 days the pile was opened and a feeding test begun with two mature susceptible rabbits. After 7 days of feeding, one animal showed a clotting time of 30 minutes as compared with 5 minutes at the beginning of the test, while the other showed 26½ minutes as compared with 6 minutes, indicating a fairly toxic sample of hay. Another larger pile (400 pounds green weight) was found to be highly toxic in a feeding test beginning 9 days after stacking. No contin-

[4] SMITH, W. K. THE ALLEGED PROTECTIVE ACTION OF ALFALFA AGAINST THE HEMORRHAGIC SWEETCLOVER DISEASE. Unpublished.

uous temperature records have been taken in piles of sweetclover spoiled under the conditions described above, but observations indicate that after 5 to 7 days a maximum temperature is reached. The pile then begins to cool. These observations suggest the relation of the heating period to the development of toxicity.

EXPERIMENTAL DATA

TESTS TO DETERMINE TOXICITY OF SPOILED MELILOTUS DENTATA AND HUBAM SWEETCLOVER

The bitter taste characteristic of common sweetclover seems not to have been reported in any other cultivated forage crop of importance. Likewise, the disease induced by the feeding of spoiled sweetclover has not been associated with the eating of any other feed. There arises therefore the possibility of some relationship between the bitter principle of common sweetclover and the development of the toxic principle in spoiled sweetclover hay. An annual form of the typically biennial *Melilotus dentata* (W. and K.) Pers., found to be nonbitter in 1933 (*1*), seemed to offer favorable material to test the existence of such a relationship. Comparisons have been made of the toxicity of spoiled hay of the annual *M. dentata* and Hubam.

A preliminary test made in 1934 was inconclusive because of the small amount of hay available and the moldy and resulting unpalatable nature of the nonbitter hay. In this trial, however, the Hubam was found to be toxic and the nonbitter to be nontoxic.

TABLE 1.—*Reaction of susceptible rabbits when fed spoiled Melilotus dentata and Hubam sweetclover hays prepared in 1935*

Kind of spoiled hay fed	Rabbit No.	Period on diet	Weight	Period on diet	Blood-coagulation time
		Days	*Grams*	*Days*	*Minutes*
Hubam	[1] 34–R–4	0	2,170	0	5
				4	12
Do	[2] 27–A–3	0	1,910	0	5
		7	1,859	4	15½
				6	34
M. dentata	17–A–5	0	2,235	0	5
		7	2,211	4	5
		14	2,235	7	6
		21	2,213	14	6
				21	5½
Do	40–R–4	0	2,262	0	5½
		7	2,192	4	6½
		14	2,125	7	5
		21	1,819	14	5
				21	6
Hubam	[3] [4] 17–A–5	0	2,213	0	5½
		5	2,203	5	13½
Hubam plus 30 g mixed grain daily	[3] [5] 40–R–4	----------	----------	0	4
				6	7
				10	18

[1] Dead on morning of the sixth day from a typical ear hemorrhage.
[2] Died on the seventh day with severe hemorrhage into the lung cavity.
[3] 17–A–5 and 40–R–4 on Hubam after a 21-day period on *M. dentata*.
[4] Died on the seventh day with severe intestinal hemorrhage.
[5] On tenth day transferred to nontoxic feed.

In the 1935 season 400 pounds (green weight) of Hubam in full bloom and a similar amount of the nonbitter annual in early bloom were cut and allowed to dry in the field until the moisture content was 52 percent for the Hubam and 47 percent for *Melilotus dentata*.

A pile of each was made in a basement laboratory. Both stacks were covered with paper, burlap, and canvas and in both vigorous heating took place. When opened for feeding after 9 days, both hays were brown with only limited amounts of mold growth. The hays were then fed ad libitum to four rabbits (two for each pile), with the results shown in table 1.

It is apparent from the data in table 1 that the Hubam sweetclover was highly toxic, inducing a significant increase in the time of blood clotting on the fourth day and killing the two rabbits (34–R–4 and 27–A–3) on the sixth and seventh day, respectively. In contrast, the blood of rabbits on the nonbitter sweetclover (*Melilotus dentata*) remained normal over a feeding period of 21 days. At the conclusion of the initial feeding period the two surviving rabbits were transferred from *M. dentata* hay to Hubam to verify their susceptibility. Of the two rabbits, 17–A–5 consumed daily during the 21-day period amounts of *M. dentata* hay comparable to that eaten by the rabbits on Hubam and maintained its weight throughout; a blood-clotting time of 15½ minutes on the fifth day of the Hubam diet and the death of this rabbit on the seventh day (table 1) demonstrate its susceptibility. On the other hand, 40–R–4 ate somewhat less *M. dentata* than the rabbits on Hubam and lost some weight during the feeding period. To compensate for this smaller consumption of nonbitter hay, when spoiled Hubam was fed, 40–R–4 received daily 30 g of mixed grain and it was estimated that by this means the amount of Hubam ingested daily was less than the daily amount of the nonbitter hay. Nevertheless, 40–R–4 showed a clotting time of 18 minutes after 10 days of feeding, indicating its susceptibility to the toxic principle.

A quantity of hay from an additional larger stack of *Melilotus dentata* sweetclover (800 pounds dry weight) which had undergone a satisfactory spoilage was fed to two additional susceptible rabbits over a 48-day period with no indication of any change in the clotting power of the blood.

In each test, the common bitter sweetclover, when spoiled, became toxic while the spoiled *Melilotus dentata* showed no indication of toxicity. Even a low level of toxicity would have been revealed since the clotting time of the blood was determined at intervals during the feeding period. There is therefore substantial evidence that the nonbitter *M. dentata* does not become toxic when the hay is stacked with a relatively high moisture content. This finding adds weight to the suggestion that the bitter principle of common sweetclover is causally related to the development of toxicity in spoiled hay.

THE RELATION OF COUMARIN TO TOXICITY

Coumarin is responsible, in part at least, for the bitter taste of common sweetclover. It has recently been shown by Brink and Roberts (*2*) that the nonbitter species, *Melilotus dentata*, does not contain this compound in measurable amount in the vegetative tissues, although small amounts were found in the seeds. Because of the absence of measurable amounts of coumarin in *M. dentata* hay and the nontoxicity of the spoiled hay, a relationship is suggested between the presence of coumarin and the production of the toxic principle in spoiled common sweetclover hay.

Coumarin apparently does not induce the sweetclover disease. Roderick and Schalk (*8*) administered coumarin in capsules to rabbits and none of the animals showed the typical symptoms of the disease. Furthermore, large numbers of cattle and other susceptible livestock when on sweetclover pasture or on well-cured sweetclover hay ingest daily considerable quantities of coumarin with apparently no effect on the clotting power of the blood. Likewise considerable quantities of spoiled alfalfa are eaten annually by cattle with no clear evidence of the occurrence of this disease. As described in detail below, coumarin was added to alfalfa, the mixture was allowed to spoil, and the resulting product was fed to rabbits.

On October 19, 1935, a lot of 500 pounds (green weight) of freshly mown third-cutting alfalfa was spread out in a greenhouse and with frequent turning was allowed to dry to approximately 50 percent moisture. It was then put through a silage cutter, and when taken to a basement laboratory was divided into two equal portions, one of which was piled up without further treatment. The other was spread out and 1 pound of coumarin ground in a ball mill was added by sifting through an 80-mesh screen; this amount gave a concentration of coumarin of 1.4 percent on the dry-weight basis—a percentage comparable to that found in sweetclover tissue. The alfalfa was turned frequently while the coumarin was being added. It was then piled up and both lots were covered with paper, burlap, and canvas. Strong heating developed rapidly in the control pile and in 3 days vigorous mold growth was apparent. The covering was removed from this material after 8 days. The alfalfa to which coumarin had been added heated more slowly, and even after 7 days no mold growth was apparent microscopically. Because of the writers' early experience of the low palatability of moldy sweetclover, the heavily molded alfalfa was not saved for feeding. In the control pile the mold infestation was so severe that the amount of mold-free hay was insufficient for a critical test of toxicity, but in the alfalfa treated with coumarin only small amounts of moldy hay were noted towards the outside of the pile. Two susceptible rabbits, fed for 10 days on the spoiled coumarin-treated alfalfa (table 2), showed clear-cut symptoms of the disease, indicating that coumarin plays a rôle in the development of toxicity.

TABLE 2.—*Reaction of two susceptible rabbits fed spoiled coumarin-treated alfalfa prepared in 1935*

Kind of hay fed	Rabbit no.	Period on diet	Blood-coagulation time
		Days	*Minutes*
Spoiled coumarin-treated alfalfa	29-A-2	0	6
		9	17½
		10	47
Do	16-A-4	0	6
		9	41
		10	60+
Well-cured alfalfa	27-A-2	0	7
		9	6
		10	7

FEEDING OF MELILOTIC ACID AND COUMARIC ACID

It has been pointed out above that coumarin does not induce the sweetclover disease but alfalfa treated with coumarin and allowed to spoil does become toxic. There is the possibility, therefore, that during spoilage coumarin is converted into a closely related compound. Two such compounds, melilotic acid and coumaric acid, which are present in relatively small amounts in sweetclover, were prepared and each was fed in capsules to two susceptible rabbits over a period of 33 days. A mixed grain and alfalfa hay were fed ad libitum. During the first 3 days each of the four rabbits received 0.1 g of compound. The amount was increased every 3 days until on the fifteenth day each received 0.75 g and by the thirtieth day, 1.5 g. The blood of the four rabbits, which was tested at intervals of a few days during this period, remained normal throughout. If all the coumarin in sweetclover tissue were converted during spoilage into melilotic acid or coumaric acid, the amount of each would rarely exceed 1 percent of the dry tissue. An animal consuming 75 g of hay daily would not absorb an amount of each compound greater than 0.75 g daily. Further, clear-cut symptoms of the disease can be detected after 5 days of feeding on a sample of sweetclover hay of moderate toxicity. One can conclude, therefore, that the amounts of melilotic acid and coumaric acid are adequate and that neither compound is directly responsible for the symptoms of sweetclover disease.

A difference was observed between the effect of coumaric and of melilotic acid on the animals. The two rabbits receiving coumaric acid had a normal appetite during the feeding period and maintained their weight, one of the rabbits at the end of the 33-day period weighing only 34 g less than at the beginning of the period, while the other weighed 17 g more. In contrast, the animals receiving melilotic acid showed signs of digestive disturbance by the fifteenth day, consuming decreasing amounts of grain ration. Alfalfa was being fed ad libitum and no weighings were made. It was apparent, however, towards the end of the feeding period, that both animals ate only the stems and refused the leafy part of the forage. One rabbit with an initial weight of 2,665 g lost 739 g in 33 days and the other with an initial weight of 2,324 g lost 452 g.

ADDITION OF MELILOTIC ACID LACTONE TO ALFALFA BEFORE SPOILAGE

The toxicity of alfalfa treated with coumarin before spoilage has already been mentioned. Since melilotic acid occurs in sweetclover in appreciable amounts and coumarin is fairly readily converted into melilotic acid in vitro, an attempt was made to determine whether melilotic acid plays a rôle in the development of the substance or substances responsible for the diminution of the prothrombin in the blood of animals afflicted with sweetclover disease. For an adequate test an amount of acid in excess of 1 pound was required. Since the lactone form can be prepared in quantity more readily than the free acid, the former was used in this experiment.

A quantity of third-cutting alfalfa was mowed on October 6 and dried down in the field to 48 percent moisture. It was then chopped in a silage cutter, taken to a basement laboratory, and put in three equal lots, each lot containing 210 pounds of partially cured hay. The first lot was left untreated; the second received 646 g of coumarin

as described in the earlier experiment; the third was treated with 655 g of melilotic acid lactone applied to the hay as a fine spray while the hay was being turned. All lots were piled and covered as earlier described. The temperature rose in the control pile more rapidly than in the other two. The control pile was likewise the first to begin cooling off. Although no temperatures were recorded during the heating period, it was estimated that all three piles heated to a similar degree. After 14 days all piles were opened and the temperature towards the center of the pile recorded; in the alfalfa plus coumarin lot, it was 42.25° C., in the alfalfa plus melilotic acid lactone 47.5°, and in the control pile 37.5°. The portions of hay having a heavy mold infestation, which was most abundant on the control pile, less on the plus lactone pile, and slight on the plus coumarin, were discarded; the remainder—with the exception of the bottom of each pile—was spread out to dry at room temperature with circulating air. When fed to susceptible rabbits, each rabbit received hay in amounts proportional to body weight. The results of the feeding tests are shown in table 3.

The absence of toxicity on the untreated but spoiled alfalfa in table 3 is in agreement with the results of farming practice wherein annually considerable quantities of brown or moldy alfalfa are fed to cattle with no indication of the symptoms of sweetclover disease. The increase in the clotting time of the blood of the two rabbits fed alfalfa treated with coumarin confirms the results of the experiment of 1935. The association of coumarin with the alfalfa tissue during spoilage in some way leads to the development of the toxic principle. There was no indication of any toxicity in the alfalfa treated with melilotic acid lactone. Although one test cannot be considered conclusive, the fact that the hay treated with lactone was part of the same lot of chopped hay used for the test with coumarin, and after treatment was kept under conditions as nearly as possible the same as the toxic coumarin-treated material, suggests that melilotic acid lactone is not a factor in the development of toxicity.

TABLE 3.—*Reaction of susceptible rabbits fed alfalfa treated with coumarin and with melilotic acid lactone prior to spoilage*

Kind of spoiled hay fed	Rabbit no.	Period on diet	Blood-coagulation time	Kind of spoiled hay fed	Rabbit no.	Period on diet	Blood-coagulation time
		Days	*Minutes*			*Days*	*Minutes*
Alfalfa alone	53-F-5	0	7½	Alfalfa plus coumarin	S-50	0	6½
		5	6½			5	16
		7	6			7	15
Do	54-F-1	0	6	Alfalfa plus melilotic acid lactone	50-S-3	0	6½
		5	6			5	6
		7	6½			7	6
Alfalfa plus coumarin	53-F-6	0	6	Do	S-48	0	6
		5	13			5	6
		7	12			7	5

DISCUSSION AND CONCLUSIONS

The widely accepted view that the characteristic bitterness of the common sweetclovers is basically attributable to coumarin has been strengthened by the discovery of a nonbitter *Melilotus* species of

which the vegetative parts are coumarin-free. It is now shown that the presence of coumarin in sweetclover has an even wider significance. Not only does the substance contribute to unpalatibility but it also gives rise to the toxic condition in spoiled hays associated with the so-called sweetclover disease in livestock.

Two lines of evidence are brought forward showing that the disease-inducing property of certain sweetclover hays results from the coumarin present in the fresh material. Recognition of *Melilotus dentata* as a sweetclover whose vegetative parts are free of coumarin provided the basis for one approach to the toxicity problem. Supplementing the evidence from this source are the results of experiments involving the addition of coumarin to partially cured alfalfa hay.

The toxicity of the various diets employed was measured by the change in time required for blood clotting in rabbits shown to be susceptible by a preliminary test.

Using hays prepared in two different years under conditions favorable to vigorous heating, direct comparisons were made between the disease-inducing capacity of an annual form of common white sweetclover and annual *Melilotus dentata*. The evidence shows that only the common sweetclover becomes toxic, the spoiled *M. dentata* hay failing to alter the blood-clotting time even with prolonged feeding. There is thus established within the genus *Melilotus* itself a parallel between the presence of coumarin and the capacity to become toxic. It would appear from these results that the absence of coumarin in the vegetative parts of *M. dentata* is the explanation both for the non-bitterness of the species and the nontoxicity of its spoiled hays.

Confirmation of this view is found in the results of further feeding experiments in which alfalfa was treated with coumarin. Roderick and Schalk (*8*) have already shown by feeding trials with rabbits that coumarin as such is not toxic, a finding which is in accord with the general observation that cattle ingesting considerable amounts of the substance on sweetclover pastures do not develop the disease. Nor is the feeding of spoiled alfalfa hay known to affect the clotting power of the blood. In the present experiments a markedly toxic preparation was obtained, however, by adding coumarin to alfalfa dried to approximately 50 percent moisture and allowing the mixture to heat. The time required for the blood to clot increased from 6 minutes to 47 and 60+ minutes, respectively, with the two rabbits fed for 10 days on this material. This result establishes a direct relationship between the presence of coumarin and the development of toxicity in a spoiling hay.

The factor immediately responsible for sweetclover disease remains to be worked out. It is shown in the present investigations that the toxicity may arise within 7 days in a vigorously heating sample of common sweetclover hay. The inference is that some change involving coumarin occurs during the fermentation period which gives rise to a substance in the hay having a destructive effect on prothrombin in the animal. Melilotic acid and coumaric acid are found to be nontoxic so that a transformation of coumarin into either of these closely related compounds during the heating period would not explain the disease-producing property of the spoiled hay. It was likewise found that the addition of melilotic acid lactone to alfalfa preceding spoilage failed to render the hay toxic. As a working hypothesis

it may be suggested that coumarin interacting with another constituent or constituents of the plant tissue common to both sweetclover and alfalfa, under moisture and temperature conditions favorable for spoilage, gives rise to a specific toxic substance.

These findings are of significance in the improvement of sweetclover as a forage plant. The evidence clearly points to the conclusion that bitterness and toxicity have a common basis in coumarin. Reduction of coumarin content by breeding or the discovery of coumarin-free forms offers the possibility not only of improving palatability but also of removing the hazard associated with the feeding of spoiled sweetclover hays.

SUMMARY

Melilotus alba, a bitter sweetclover, on being stacked at about 50 percent moisture and allowed to heat, gives a hay which, when fed to rabbits, induces a condition characteristic of the so-called sweetclover disease in cattle by markedly lowering the clotting power of the blood.

Parallel tests with *Melilotus dentata*, a sweetclover which has recently been recognized as being nonbitter, show that this species does not become toxic on being similarly spoiled.

Neither spoiled alfalfa hay nor coumarin appears to modify the clotting power of the blood. If, however, coumarin is mixed with partially cured alfalfa hay containing about 50 percent moisture, the mixture on being allowed to heat becomes distinctly toxic.

Melilotic acid and coumaric acid, compounds closely related to coumarin, are like the latter substance in that they do not induce sweetclover disease on being fed to rabbits.

In one test melilotic acid lactone added to alfalfa before spoilage, contrary to the behavior of coumarin, did not induce toxicity.

It is tentatively suggested that coumarin interacting with another constituent or constituents of the plant tissue, under conditions favorable for spoilage, gives rise to a specific toxic substance which is responsible for the sweetclover disease in animals.

Since coumarin appears to be a basic factor in both bitterness and toxicity, the development of nonbitter forms of sweetclover gives promise not only of improving the palatability of this plant but also of eliminating the hazard associated with the feeding of improperly cured hay.

LITERATURE CITED

(1) Brink, R. A.
1934. A NON-BITTER VARIETY OF MELILOTUS. Science 79: 301.
(2) ——— and Roberts, W. L.
1937. THE COUMARIN CONTENT OF MELILOTUS DENTATA. Science 86: 41–42.
(3) Brown, J. M., Savage, A., and Robinson, A. D.
1933. A REPORT OF SOME INVESTIGATIONS INTO THE CAUSE OF SWEET CLOVER (MELILOTUS) DISEASE. Sci. Agr. 13: 561–565.
(4) Cannon, C. Y., and Greenwood, Delbert.
1930. EFFECT OF A DIET OF SWEET CLOVER ON THE CALCIUM IN THE BLOOD SERUM. Jour. Dairy Sci. 13: 424–431.
(5) Quick, Armand J.
1936. THE COAGULATION DEFECT IN PEPTONE SHOCK AND IN SWEET CLOVER DISEASE. Jour. Biol. Chem. 114: LXXXII.
(6) ——— Stanley-Brown, M., and Bancroft, F. W.
1935. A STUDY OF THE COAGULATION DEFECT IN HEMOPHILIA AND IN JAUNDICE. Amer. Jour. Med. Sci. 190: 501–511.

(7) RODERICK, LEE M.
1931. A PROBLEM IN THE COAGULATION OF THE BLOOD. "SWEET CLOVER DISEASE OF CATTLE." Amer. Jour. Physiol. 96: 413–425, illus.
(8) ——— and SCHALK, A. F.
1931. STUDIES ON SWEET CLOVER DISEASE. N. Dak. Agr. Expt. Sta. Bull. 250, 56 pp., illus.
(9) SCHOFIELD, FRANK W.
1922. A BRIEF ACCOUNT OF A DISEASE IN CATTLE SIMULATING HEMORRHAGIC SEPTICAEMIA DUE TO FEEDING SWEET CLOVER. Canad. Vet. Rec. 3: 74–78.
(10) ———
1924. DAMAGED SWEET CLOVER: THE CAUSE OF A NEW DISEASE IN CATTLE SIMULATING HEMORRHAGIC SEPTICEMIA AND BLACKLEG. Jour. Amer. Vet. Med. Assoc. 64: 553–575.

CYTOLOGY OF TWIN COTTON PLANTS[1]

By J. M. WEBBER

Associate cytologist, Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The occurrence of polyembryonic cottonseeds was first reported by Harland (*3*).[2] He found that seeds with two embryos occur sporadically in several strains of cultivated American cottons. Of such pairs of embryos, some gave rise to pairs of identical diploids; others to pairs consisting of one haploid and one diploid. In sea-island cottons Harland raised 16 pairs to maturity, of which 14 were haploid-diploid twins. Harland also reported haploid-diploid twins in upland cottons and in strains of *Gossypium hirsutum* × *G. barbadense* ancestry.

Harland found that haploids from particular strains of sea-island cottons were somewhat female-fertile. One haploid was unusually fertile when pollinated with pollen from Asiatic and wild American cottons, as well as with pollen from normal sea-island cotton. Other sea-island and upland haploids were found to be sterile. None of the haploids formed viable pollen.

The writer is unaware of any reports upon the cytology of cotton twins. Statements concerning the cytology of cotton haploids appear to be limited to the following: (1) Harland's (*2*) mention of Skovsted's discovery that "man cotton" is a haploid possessing only 26 chromosomes in somatic cells, (2) Skovsted's (*4*) report of a few trisomes in a haploid New World cotton, and (3) Harland's (*3*) statement to the effect that crosses of 13-chromosome Asiatic or of 13-chromosome wild American species with both haploid and diploid sea island are apparently identical. Such phenotypic expression, in conjunction with the relatively high female fertility of particular haploids, probably indicates that these haploids form a considerable number of unreduced gametes with 26 chromosomes.

OBSERVATIONS

TYPES OF TWINS

At the Rubidoux Laboratory, Riverside, Calif., within the past 3 years, four polyembryonic cottonseeds have been recorded during germination experiments. Each seed gave rise to two seedlings. The seedlings proved to be two pairs of diploid twins, one pair of which was conjoined; one pair of haploid-diploid twins; and one a diploid, the twin of which died in the seedling stage before cytological material could be obtained.

[1] Received for publication October 5, 1937; issued August 1938.

[2] Italic numbers in parentheses refer to Literature Cited, p. 160.

DIPLOID TWINS

One pair of identical diploid twins was derived from an extra large seed occurring in third-generation selfed seeds of the red variation of Acala cotton (*Gossypium hirsutum* L., No. W64). The twins were raised to maturity. They proved to be practically indistinguishable from each other and their parent. Likewise the meiotic chromosome behavior of the twins and their parent was nearly identical and similar to that of other species of cultivated American cottons (*6*). Although the twins generally formed 26 bivalent chromosomes during the first metaphase, occasionally 1 or 2 and rarely 3 quadrivalents occurred. First anaphase disjunction was normal, except for an occasional lagging bivalent. Second metaphase plates usually contained 26 chromosomes, though rarely one plate of a microspore with 1 or 2 chromosomes less than the haploid number occurred.

The second pair of diploid twins occurred in an F_2 generation of a

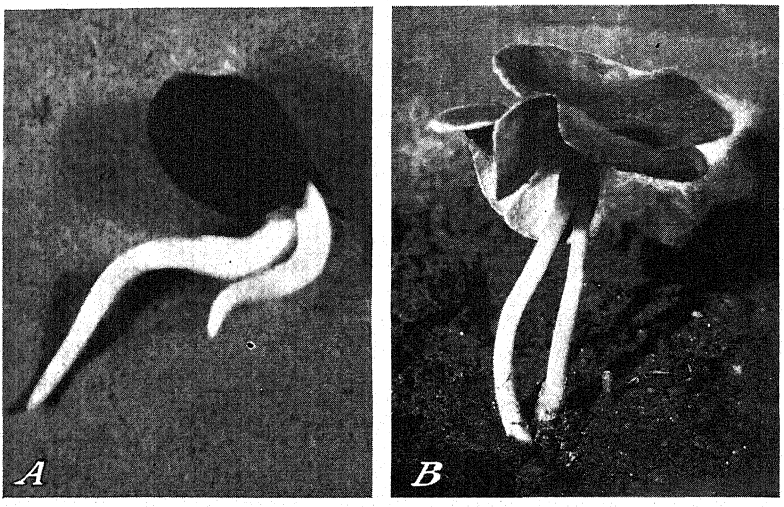


FIGURE 1.—*Gossypium nanking* × *G. thurberi*, F_2, conjoined diploid twins: *A*, Germinated seed with two radicles; *B*, young seedlings with conjoined cotyledons and hypocotyls. × 4.

hybrid between *Gossypium nanking* Meyen ($n=13$) and *G. thurberi* Tod. ($n=13$). The parent of these twins formed an average of 25.6 univalents and 0.2 bivalent chromosomes (*7*) during meiosis. It was practically sterile, forming only two open-pollinated or parthenogenic seeds. Upon germination, one of these seeds formed two radicles (fig. 1, *A*). In the cotyledon stage (fig. 1, *B*), it was evident that the seed had given rise to conjoined twins. Each of these twins had one cotyledon and shared the third, which was slightly below the other two. Their hypocotyls were united at the base of the two upper cotyledons and for fully a quarter of an inch toward the roots. As the twins grew, two buds were formed, one at the base of each free cotyledon. The buds developed into shoots approximately 2 inches long with several leaves, when the roots of the twins became diseased and the plants died.

A cytological examination of the root tips of the twins during the cotyledon stage revealed that each possessed 26 somatic chromosomes.

HAPLOID-DIPLOID TWINS

The haploid-diploid twins occurred in a culture of *Gossypium barbadense* L. (C. B.[3] No. 1065). In external appearance the seed giving rise to the twins was identical with seeds that produced only a single plant. The polyembryonic nature of the germinating seed was not determinable until the diploid twin had grown well above the soil level and its cotyledons had unfolded. At this stage of development, a second radicle appeared between the cotyledons (fig. 2, *A*), which proved to belong to a second plant. This plant was later determined to be a haploid.

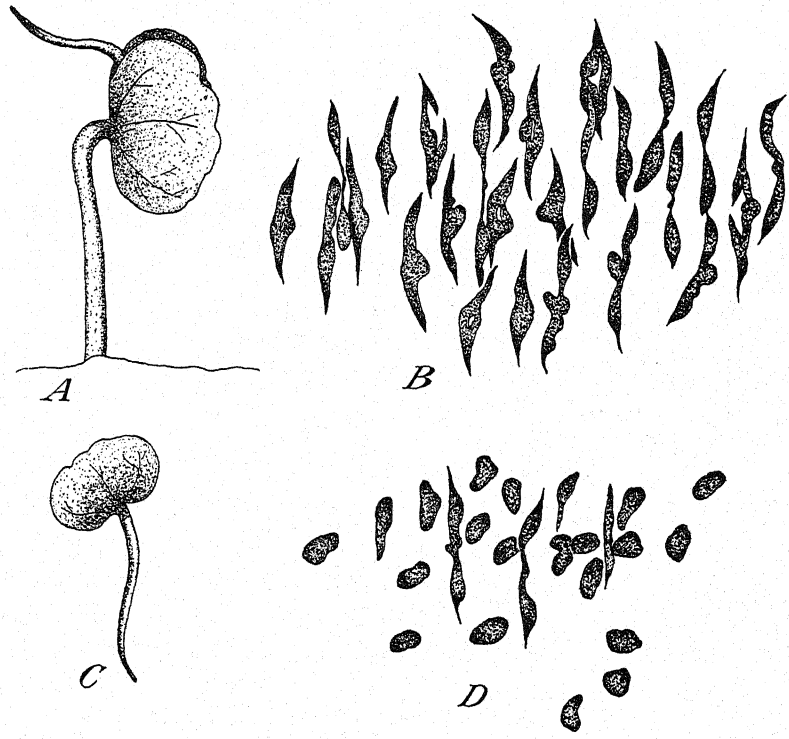


FIGURE 2.—*Gossypium barbadense*, haploid-diploid twins. *A*, Diploid twin with radicle of haploid protruding between cotyledons. × 1⅛. *B*, Diploid twin: First metaphase in pollen mother cell; 26 bivalent chromosomes. × 3,375. *C*, Haploid twin. × 1⅛. *D*, Haploid twin: First metaphase in pollen mother cell; 2 bivalent and 22 univalent chromosomes. × 3,375.

The haploid twin (fig. 2, *B*) differs from the diploid chiefly in its dwarfed stature, smaller leaves and flowers, and reduced fertility. It has been maintained for 3 years, during which it has set only three open-pollinated and one selfed seed. Attempts to produce reciprocal crosses between it and several normal cultivated American cottons have been unsuccessful.

The types of meiotic association occurring in the haploid and diploid twins and in the selfed offspring of the former are summarized in table 1. In figures 2, *B*, and 2, *D*, are shown, respectively, first metaphases of the diploid twin having 26 bivalent chromosomes, and

[3] Cotton breeding number.

of the haploid twin having 2 bivalent and 22 univalent chromosomes. Out of a group of 35 second-division pollen mother cells of the haploid, only 2 exhibited a failure of the reduction division. Each of these two cells had a single metaphase plate containing 26 chromosomes. Of the highly irregular sporads, 46 percent were tetrads and 6.6 percent diads.

The significant meiotic features are the limited chromosome pairing in the haploid, the occurrence of quadrivalents in its diploid twin, and the similarity of type of chromosomal association in the diploid twin and haploid offspring.

TABLE 1.—*First metaphase chromosome association in pollen mother cells of Gossypium barbadense*

Haploid (1065-*n*)			Diploid [1] (1065)			Diploid [2] (1065-2*n*)		
Association [3]		Pollen mother cells	Association [3]		Pollen mother cells	Association [3]		Pollen mother cells
Type	Mean		Type	Mean		Type	Mean	
		Number			*Number*			*Number*
$0_{II}+26_{I}$--	$0.2_{II}+25.6_{I}$-	22	$26_{II}+0_{I}$------	$25.66_{II}+0.12_{I}+0.14_{IV}$	44	$26_{II}+0_{I}$------	$25.64_{II}+0.16_{I}+0.14_{IV}$	42
$1_{II}+24_{I}$--		1	$25_{II}+2_{I}$------		2	$25_{II}+2_{I}$------		3
$2_{II}+22_{I}$--		2	$22_{II}+2_{IV}$-----		3	$24_{II}+1_{IV}$-----		2
			$23_{II}+2_{I}+1_{IV}$-		1	$22_{II}+2_{IV}$-----		2
						$23_{II}+2_{I}+1_{IV}$-		1

[1] Twin of haploid.
[2] Offspring of selfed haploid.
[3] The subscripts I, II, and IV are cytological symbols designating univalent, bivalent, and quadrivalent chromosomes, respectively.

The fourth pair of twins occurred in the same culture as the preceding haploid-diploid twins. The germinating seed produced two radicles, similar to those of the identical twins depicted in figure 1, *A*. One of the twins died in the seedling stage and no further study was made, except to determine that the remaining plant was a normal diploid.

DISCUSSION

Harland (*3*) has suggested that in plants originating from unlike chromosome sets polyembrony might arise as a primitive character brought into expression by genic disharmony. His suggestion is based on the fact that polyembryony in cottons has previously been found to occur only in cultivated American species that are possibly allopolyploids. All except one of the four pairs of twins recorded in the present report occurred in cultivated American cottons. That genic disharmony occurred in the parent of the exceptional pair of twins (*Gossypium nanking* × *G. thurberi*) is shown by the fact that it exhibits very little meiotic chromosome conjugation and is practically sterile. It is also noteworthy that the parental culture of *Gossypium barbadense* (C. B. No. 1065), in which occurred two of the twins recorded here, is a decidedly weak strain and somewhat sterile.

Harland further suggested that in polyembryonic cottons the occurrence of haploid-diploid twins indicates the development of two embryos, one "from an unfertilized egg alongside its fertilized neigh-

bor." This suggestion is supported by the cytological observations of Baranov (*1*), who previously reported that *Gossypium herbaceum* frequently forms ovules containing more than one embryo sac. Hence, in cases where the meiotic divisions of the parent are normal and viable pollen and eggs are formed, Harland's explanation of the origin of haploid-diploid and diploid twins is the most logical. However, in the formation of diploid *G. nanking* × *G. thurberi* twins, it appears very unlikely that 13-chromosome twin eggs should be formed and fertilized with 13-chromosome sperms. In this case it is more likely that the production of the twins involved sporophytic budding or diploid parthenogenesis.

Harland (*3*) pointed out that in cultivated cottons the degree of heterozygosity is so great that no pure lines have ever been produced. To obtain approximately homozygous strains, he suggested the use of haploids and stated: "If it were possible * * * to produce diploid shoots on the cotton haploids, we should have, ab initio, a pure line." As an alternative method of approaching homozygosity he suggested repeated matings of a standard haploid with its successive progenies of haploid × diploid. That neither of these methods is likely to produce a pure line is indicated by the chromosome pairing in the haploid and the quadrivalent formation in its selfed offspring, here reported. Observations of Skovsted (*4*, *5*) and Webber (*6*) have shown that at least four chromosomes within the haploid set of all cultivated American cottons are to some extent cytologically homologous. It is possible that these chromosomes cause haploids to be partly heterozygous and add to the complexity of the heterozygotes.

The limited pairing of chromosomes within the haploid, here reported, strongly supports Skovsted's (*5*) conclusion that cultivated American cottons are allopolyploids.

SUMMARY

The following polyembryonic plants are described: (1) Diploid twins of *Gossypium hirsutum*, (2) conjoined diploid twins of *G. nanking*×*G. thurberi* F_2, and (3) haploid-diploid twins of *G. barbadense*.

The cytology of the twins supports the suggestion that polyembryony is an expression of genic disharmony between two unlike sets of chromosomes.

The twins of *G. hirsutum* and *G. barbadense* probably developed from twin eggs, while those of *G. nanking*×*G. thurberi* F_2, probably involved sporophytic budding.

The mean chromosome conjugation of haploid *G. barbadense* was 25.6 univalents plus 0.2 bivalent; of its diploid twin, 0.12 univalent plus 25.66 bivalents plus 0.14 quadrivalent; and of its selfed offspring, 0.16 univalent plus 25.64 bivalents plus 0.14 quadrivalent.

Reference is made to the possibility of obtaining homozygous lines from haploids.

Limited pairing of chromosomes in haploid *G. barbadense* supports the suggestion that cultivated American cottons are allopolyploids.

LITERATURE CITED

(1) BARANOV, P.
1930. COTTON PLANT: CYTOLOGY. PLANT BREEDING. Bull. Sci. Research Cotton Inst. Tashkent 5: 7–17. [Abstract in Plant Breeding Abs. 2: 197–198. 1932. Original not seen. Abstract in Jour. Textile Inst. 23: A475. 1932.]

(2) HARLAND, S. C.
1932. THE GENETICS OF GOSSYPIUM. Bibliographia Genetica 9: [107]–178.

(3) ———
1936. HAPLOIDS IN POLYEMBRYONIC SEEDS OF SEA ISLAND COTTON. Jour. Heredity 27: 229–231.

(4) SKOVSTED, A.
1933. CYTOLOGICAL STUDIES IN COTTON. I. THE MITOSIS AND MEIOSIS IN DIPLOID AND TRIPLOID ASIATIC COTTON. Ann. Bot. [London] 47: [227]–251, illus.

(5) ———
1934. CYTOLOGICAL STUDIES IN COTTON. II. TWO INTERSPECIFIC HYBRIDS BETWEEN ASIATIC AND NEW WORLD COTTONS. Jour. Genetics 28: [407]–424, illus.

(6) WEBBER, J. M.
1934. CHROMOSOME NUMBER AND MEIOTIC BEHAVIOR IN GOSSYPIUM. Jour. Agr. Research 49: 223–237, illus.

(7) ———
1936. CYTOGENETIC NOTES ON COTTON AND COTTON RELATIVES, II. Science 84: 378.

U. S. GOVERNMENT PRINTING OFFICE: 1938

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 57 WASHINGTON, D. C., AUGUST 1, 1938 NO. 3

MOISTURE MOVEMENT IN WOOD ABOVE THE FIBER-SATURATION POINT[1]

By LOUIS W. REES, *assistant forester, Minnesota Agricultural Experiment Station,* and STANLEY J. BUCKMAN, *head of research department, American Creosoting Co.*

INTRODUCTION

Free water normally is present in wood at the beginning of the air-seasoning process, and also when wood is kiln-dried from the green or in some cases from the partly air-seasoned condition. As long as the wood contains free water in the cell cavities the movement of moisture to the surface may take place in a number of different ways. It may move as free water, as bound liquid, as vapor, or as a result of the combined action of any or all of these three possible mechanisms.

Because of the elimination of the possibility of free water movement in wood below the fiber-saturation point, the interpretation of the results obtained under these conditions is somewhat simplified. Several studies have been made recently of moisture movement through wood in which no free water was present. Martley (*15*)[2] and Stillwell (*26*) have investigated the movement of moisture through wood under conditions producing a more or less constant rate of movement. This was done by maintaining a constant high relative humidity at one end of the block and a constant low relative humidity at the opposite end in conjunction with methods which served to limit the moisture flow to one structural direction of the block. The results obtained did not indicate definitely the mechanism of moisture movement. Martley's analysis of his data showed, however, that a more rapid rate of moisture movement was obtained in the radial direction of Scotch pine with increasing moisture content of the wood below the fiber-saturation point. Stillwell's results indicated that the rates of moisture movement in the longitudinal direction of oak and ash varied from about one and one-half to seven times those in the radial and tangential directions.

Pidgeon and Maass (*16*) have studied the movement of moisture into small blocks composed entirely of the sapwood or heartwood of white spruce. These blocks were dried by prolonged evacuation in the presence of phosphorous anhydride and allowed to regain moisture at a vapor pressure of 4.58 mm of mercury and a temperature of 23° C. When the moisture movement was confined to the longitudinal direction, it was found that the time to half saturation for the heartwood varied from about two and one-fourth to seven and one-half times that for the sapwood; while for radial movement in the heartwood of white spruce the time to half saturation was about two and one-half times that for the sapwood. Although direct comparisons were not

[1] Received for publication November 19, 1937; issued August 1938. Paper No. 1526, Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] Italic numbers in parentheses refer to Literature Cited, p. 186.

possible because the moisture movement did not take place through the same thickness of wood, the results further indicated that the rate of moisture movement in the radial direction was at least 20 times that in the longitudinal direction for both the heartwood and sapwood of white spruce. Pidgeon and Maass concluded from these results that moisture moves in wood below the fiber-saturation point in the form of vapor. However, since Pidgeon and Maass subjected evacuated wood to a definite vapor pressure in the absence of air their results are comparable only to pressure-permeability data and do not necessarily represent the relative rates of moisture diffusion to be expected under the conditions normally employed in seasoning wood.

In a recent paper, Buckman and Rees (*1*) concluded that moisture moves in wood below the fiber-saturation point predominantly by means of a mechanism involving the movement of the moisture across the cell cavities in the form of vapor and through the walls in the form of bound water. Evidence to support this conclusion was found in the fact that a definite relationship existed between the time required to gain half-saturation moisture content and the specific gravity for both sapwood and heartwood of different kinds of wood. Within the limits of the range of the specific gravity of the woods studied, this relationship appeared to be linear. However, other unpublished data collected by these authors showed that a straight-line relationship does not hold throughout the entire range of the specific gravity of wood, but that the relationship takes the form of an exponential function. The authors pointed out and discussed the reasons why such a relationship, when considered in the light of other available information, supports the predominant operation of a dual mechanism consisting of vapor movement across the cell cavities and bound liquid movement through the cell walls, in contrast to movement predominantly either in the form of vapor or bound liquid alone.

From theoretical calculations, using the approximate diameter and length of a softwood tracheid, the approximate thickness of the cell wall, and the relative rates of movement in the radial and tangential directions as compared to the longitudinal direction, Buckman and Rees (*1*) found that the relative rates of moisture movement as a vapor across the cell cavities and as a bound liquid through the cell walls were of the approximate order of magnitude of 100 to 1.

Tuttle (*28*), Sherwood, and coworkers (*3*, *8*, *18*, *19*, *20*, *21*), and Luikov (*13*) have used the diffusion equation to calculate the rate of moisture movement through wood and other solids when these materials contained free water. A good agreement was found between the calculated and experimental results. However, as Hawley (*11*) has stated, this agreement does not establish that the mechanism of moisture movement in wood corresponds to the assumptions of simple diffusion. Hawley has pointed out that, on the contrary, free water would create no potential which would cause it to diffuse from a higher to a lower concentration. Hawley explains the evidence of free water movement during the drying of some woods on the basis of a quite different mechanism involving the capillary forces which are set up in the cell cavities and the openings through the pit membranes during the drying process.

Although considerable work has already been done on the movement of moisture through wood below the fiber-saturation point, this information is not necessarily directly applicable to the seasoning of

wood as it is done in actual practice, since green wood always contains free water which must be removed during the seasoning process. The purpose of the present paper is to present: (1) Data on the relative rates of moisture movement in the different structural directions of heartwood and sapwood of different woods containing a maximum amount of free water at the beginning of the drying process, and (2) a discussion of these data in terms of the predominant mechanism of moisture movement in wood above the fiber-saturation point.

MATERIALS

Six different kinds of hardwoods and one softwood were used in the experiments. These woods were red oak (*Quercus borealis* Michaux f.), northern bur oak (*Q. macrocarpa var. olivaeformis* (Michaux f.), Gray), bitternut hickory (*Hicoria cordiformis* (Wangenheim) Britton), basswood (*Tilia glabra* Ventenat), silver maple (*Acer saccharinum* Linnaeus), hackberry (*Celtis occidentalis* Linnaeus), and red pine (*Pinus resinosa* Aiton).

The blocks used in the experiments, 2.5 by 2.5 by 5.0 cm in size, were sawed from green logs. The history of the material from which the blocks were obtained was known from the time the trees were felled until the wood was used. The blocks were cut to conform to the three structural directions of the wood and whenever possible samples were secured from both the heartwood and the sapwood of the same log.

METHODS

Immediately after the blocks were prepared, they were slowly dried to equilibrium with atmospheric conditions in the laboratory and maintained under these conditions until used in the experiment. Four blocks then were selected representing each structural direction of each of the hardwoods, and five blocks were selected representing each structural direction of the one coniferous wood used in the study. Blocks representing both the heartwood and the sapwood were selected whenever available. In addition, a representative block was selected with each group for preliminary moisture determinations. The oven-dry weight of each group of four or five blocks then was calculated, assuming that the group contained the same moisture content as the selected block.

The blocks representing the sapwood and heartwood of each different kind of wood then were placed in a separate container filled with tap water and subjected alternately to a vacuum of about 70 cm of mercury and pressure of 3.5 kg per square centimeter for a period of about 3 weeks. The water on the specimens was changed frequently in order to prevent a possible development of fungi or bacteria in the water. After the blocks had been treated in this way for about 1 week, the green volume of each set of blocks was determined by the water-immersion method. From the data thus accumulated, the theoretical maximum amounts of moisture which the blocks could absorb was calculated by the formula

$$M = 100\left(\frac{1}{S} - \frac{1}{1.55}\right)$$

in which M is maximum percentage of moisture in the wood when all of the air space is completely filled with water, S is the specific gravity of the wood based on the calculated oven-dry weight and the volume

of the wood at a moisture content above the fiber-saturation point, and 1.55 is the density of wood substance. Soaking of the blocks in the manner described above was continued until they had reached approximately these calculated maximum moisture contents.

When the blocks were saturated, they were removed from the water, wiped with a damp cloth, and allowed to stand in the atmosphere of the laboratory for about 15 minutes in order to remove most of the surface moisture. They then were coated on five surfaces with 16 coats of vulcanized rubber latex. The end of each block, which was to be exposed later, was covered during the coating process with a sheet of tinfoil, in order to retard evaporation at the uncoated end. After coating, the blocks were returned to the water with the tinfoil

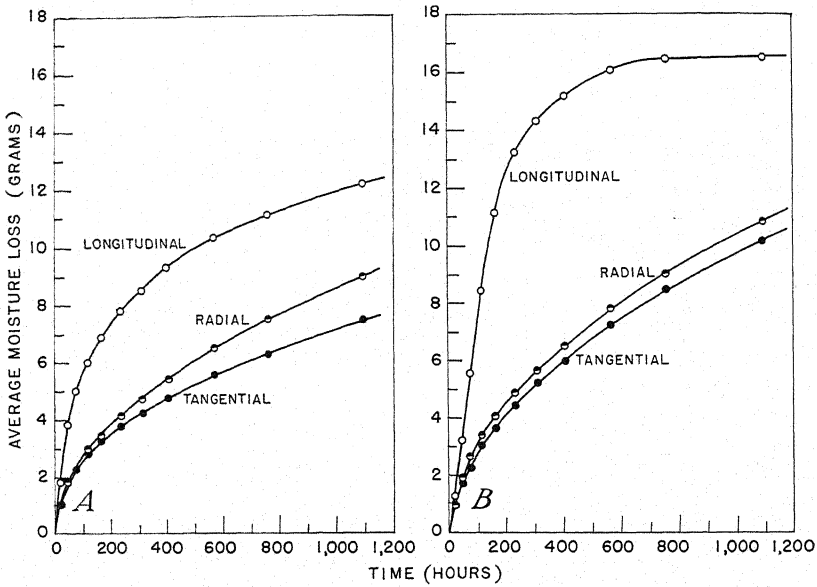


FIGURE 1.—Average moisture loss in grams of blocks of red oak heartwood (*A*) and red pine heartwood (*B*) when held at 30° C. and 81 percent humidity, the moisture movement having been confined to the three structural directions.

still attached and stored in this manner until the start of the experiment. At this time, the blocks were removed and allowed to stand in the laboratory for at least 2 hours in order that evaporation of water from the surface of the rubber coating could take place. The tinfoil then was removed and a rubber band placed around the block near the exposed end in order to prevent the coating from coming loose at this place. Each group of blocks was weighed to the nearest 0.01 and then placed in a cabinet maintained at a temperature of 30° C. and a relative humidity of 81 percent. At the end of 19.5 hours, the groups of blocks were weighed again and the decrease in weight was calculated. The weighings were repeated thereafter at what appeared to be suitable intervals of time. The entire series of blocks were run at the same time, thereby more or less eliminating the influence of any minor fluctuations in temperature and humidity on the relative nature of results obtained.

Immediately after the last weights were taken, the rubber coatings were removed and the blocks weighed, the weights of the coatings

being obtained by a subtraction of the results of these two determinations. The oven-dry weights were determined after the blocks had been dried to a constant weight at atmospheric pressure and a temperature of 105° C. From these data, the specific gravities based on the oven-dry weight and green volume were calculated. The dimensions of the surface exposed during the drying process were determined from the resoaked blocks after they had been oven-dried.

EXPERIMENTAL RESULTS

The data showing the average moisture loss in grams with time for the groups of blocks employed in investigating the moisture movement in the three structural directions of the different kinds of wood are given in table 1. Representative curves showing these data graphically are given in figure 1.

TABLE 1.—*Average total moisture loss in grams when the moisture movement was confined to the three structural directions of the different woods*

Kind of wood and direction	Average moisture loss [1] when the elapsed time was—										
	19.5 hours	44 hours	74 hours	116 hours	165 hours	236 hours	312 hours	408 hours	572 hours	764 hours	1,100 hours
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Red oak heartwood:											
Longitudinal	1.85	3.86	5.03	6.06	6.86	7.78	8.51	9.53	10.36	11.17	12.22
Radial	1.13	1.84	2.39	2.98	3.49	4.16	4.75	5.45	6.51	7.52	9.03
Tangential	1.08	1.75	2.24	2.81	3.27	3.79	4.24	4.74	5.53	6.29	7.46
Bur oak heartwood:											
Longitudinal	1.33	3.84	5.68	7.18	8.15	9.16	9.89	10.70	11.70	12.41	13.30
Radial	1.00	1.67	2.22	2.80	3.37	4.08	4.74	5.51	6.59	8.07	9.24
Tangential	.96	1.63	2.14	2.76	3.33	4.09	4.76	5.53	6.64	8.51	9.24
Hickory heartwood:											
Longitudinal	1.55	3.79	5.28	6.55	7.38	8.25	8.84	9.43	10.17	10.74	11.41
Radial	.92	1.61	2.11	2.66	3.17	3.84	4.37	4.98	5.95	6.83	8.13
Tangential	.82	1.42	1.78	2.28	2.66	3.17	3.60	4.12	4.84	5.54	6.55
Basswood heartwood:											
Longitudinal	1.55	4.21	6.77	10.93	14.92	18.45	19.57	20.49	21.08	21.29	21.35
Radial	1.49	3.99	6.15	8.22	9.50	10.89	12.03	13.19	14.78	16.19	18.24
Tengential	1.76	4.53	7.07	10.42	13.42	16.54	17.82	18.65	19.52	20.07	20.55
Silver maple heartwood:											
Longitudinal	1.48	3.96	6.53	10.30	13.04	15.43	16.69	17.41	18.12	18.50	18.73
Radial	1.10	2.28	3.11	4.33	4.88	5.94	6.86	7.93	9.52	11.10	13.21
Tangential	1.02	1.81	2.35	3.02	3.55	4.28	4.90	5.58	6.59	7.55	8.73
Silver maple sapwood:											
Longitudinal	1.29	3.76	6.08	9.70	11.70	13.62	14.81	15.66	16.45	16.97	17.17
Radial	1.55	3.87	5.44	7.21	8.57	10.20	11.55	12.90	14.45	15.52	16.44
Tengential	1.56	3.43	4.48	5.60	6.35	7.23	7.81	8.45	9.37	10.21	11.44
Hackberry heartwood:											
Longitudinal	2.29	3.45	5.37	7.84	9.40	10.68	11.50	12.25	13.04	13.70	14.33
Tangential	1.49	2.05	2.74	3.50	4.09	4.83	5.45	6.11	7.03	7.93	9.20
Hackberry sapwood:											
Longitudinal	1.46	3.61	5.83	8.62	10.89	12.79	14.07	15.25	16.44	17.23	17.86
Tangential	1.18	2.08	2.77	3.57	4.26	5.14	5.92	6.80	8.06	9.28	11.00
Red pine heartwood:											
Longitudinal	1.28	3.23	5.55	8.46	11.14	13.27	14.31	15.19	16.07	16.44	16.48
Radial	.94	1.87	2.60	3.41	4.07	4.90	5.64	6.48	7.74	9.01	10.80
Tangential	.92	1.68	2.29	3.03	3.65	4.45	5.18	5.98	7.20	8.40	10.12
Red pine sapwood:											
Longitudinal	1.83	3.55	5.52	8.06	10.49	13.38	15.52	16.65	17.28	17.50	17.52
Radial	1.67	3.98	5.80	7.67	9.17	10.84	12.29	13.82	15.79	16.91	17.69
Tangential	1.07	1.94	2.61	3.40	4.08	4.93	5.71	6.63	8.01	9.40	11.30

[1] Zero loss at zero hours.

After curves, similar to those shown in figure 1, had been prepared for all of the woods used in the experiments, the rates of moisture loss in grams per hour were determined for selected points on each curve from mechanically constructed lines having the same slope as the tangents at these points. These lines were constructed by erecting a perpendicular to the tangent for each selected point on the curve with

the aid of a plateglass mirror about 6 inches long and three-quarters of an inch high. One edge of the mirror was ground so that the reflecting surface would stand at right angles to the paper on which the curves were drawn. A line having the same slope as the tangent to the curve

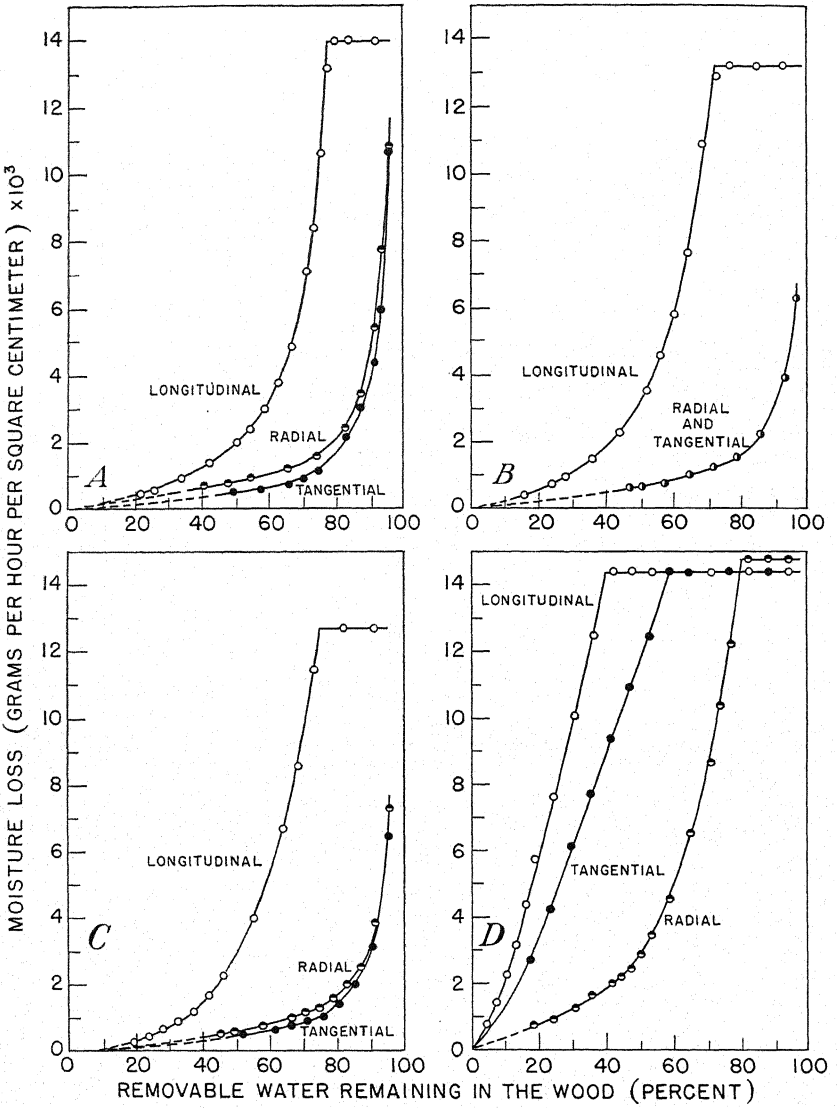


FIGURE 2.—Relation between the rate of moisture loss and the percentage of removable water remaining in the wood for the different structural directions of (*A*) red oak heartwood; (*B*) bur oak heartwood; (*C*) hickory heartwood; (*D*) basswood heartwood.

at the point of intersection of the perpendicular and the curve then was constructed by drawing a line at right angles to this perpendicular.

The rates of moisture loss in grams per hour obtained by the above method were divided by the average green cross-sectional areas of the groups of blocks in order to determine the rates of moisture loss in

grams per hour per square centimeter of exposed surface. Shrinkage was not taken into account in calculating the rates of moisture loss when a portion of the blocks was below the fiber-saturation point. However, the magnitude of the difference in amount of shrinkage for

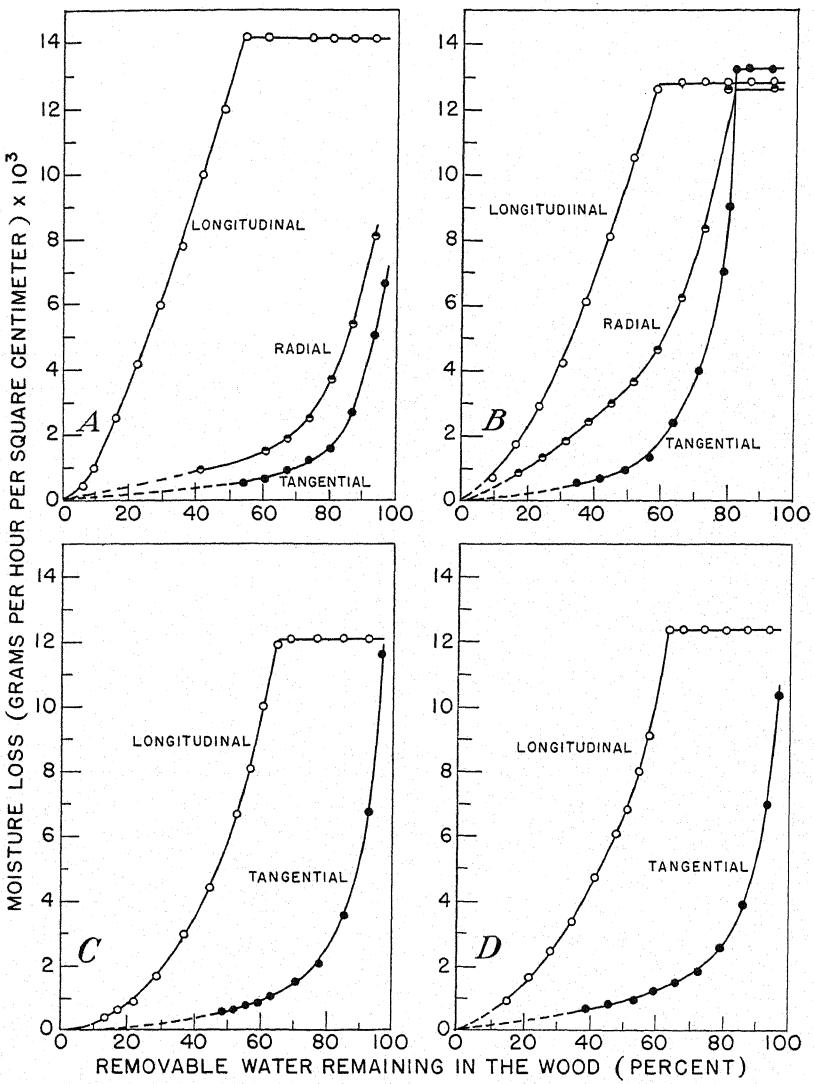


FIGURE 3.—Relation between the rate of moisture loss and the percentage of removable water remaining in the wood for the different structural directions of (*A*) silver maple heartwood; (*B*) silver maple sapwood; (*C*) hackberry heartwood; (*D*) hackberry sapwood.

the different woods when dried to an equilibrium moisture content of about 16 percent, is comparatively small. The percentage of total removable water which still remained in the blocks then was calculated in the case of each point for which the rate of moisture loss was determined. The data obtained by the foregoing procedure are shown graphically in figures 2, 3, and 4.

From curves given in figures 2, 3, and 4, the following data were obtained: (1) The rates of moisture loss during the constant rate period, (2) the percentages of total removable water remaining in the wood at the critical point, which will be referred to in the subsequent discussion as "the critical moisture content," and (3) the rates of moisture loss when 50 and 25 percent of the total removable water still remained in the wood. These data, the specific gravity of the various woods, and the ratios between the rates of moisture loss for movement in the different structural directions of the different woods when 50 and 25 percent of the total removable water still remained in the wood, are given in table 2.

TABLE 2.—*Summary of the results for moisture movement in the 3 structural directions of the different kinds of wood*

Kind of wood	Specific gravity			Water loss per hour per square centimeter of exposed surface during the constant rate period			Percentage of total removable water in the wood at the critical moisture content			Water loss per hour per square centimeter of exposed surface when 50 percent of the removable water remained in the blocks		
	Longitudinal	Radial	Tangential	Longitudinal	Radial	Tangential	Longitudinal	Radial	Tangential	Longitudinal	Radial	Tangential
				Grams	*Grams*	*Grams*	*Pct.*	*Pct.*	*Pct.*	*Grams*	*Grams*	*Grams*
Red oak heartwood	0.620	0.605	0.581	0.01399	(1)	(1)	77.5	(1)	(1)	0.00195	0.00083	0.00050
Bur oak heartwood	.575	.560	.590	.01320	(1)	(1)	72.0	(1)	(1)	.00341	.00063	.00063
Hickory heartwood	.632	.654	.643	.01270	(1)	(1)	75.0	(1)	(1)	.00288	.00058	.00043
Basswood heartwood	.367	.357	.375	.01440	0.01480	0.01440	40.0	80.0	59.0	.01440	.00286	.01170
Silver maple heartwood	.437	.448	.475	.01420	(1)	(1)	54.2	(1)	(1)	.01275	.00113	.00050
Silver maple sapwood	.493	.477	.468	.01280	.01260	.01320	58.7	81.5	81.5	.01000	.00348	.00099
Hackberry heartwood	.550	(2)	.528	.01210	(1)	(1)	64.8	(1)	(1)	.00577	(2)	.00057
Hackberry sapwood	.468	(2)	.474	.01237	(1)	(1)	63.3	(1)	(1)	.00667	(2)	.00086
Red pine heartwood	.361	.363	.374	.01173	(1)	(1)	48.0	(1)	(1)	.01173	.00090	.00068
Red pine sapwood	.412	.405	.416	.01110	.01045	(1)	50.0	70.0	(1)	.01110	.00442	.00112
Average	-----	-----	-----	.01285	.01262	.01380	-----	-----	-----	-----	-----	-----

Kind of wood	Comparative rates of moisture loss in the different structural directions, when 50 percent of the removable water remained in the block			Water loss per hour per square centimeter of exposed surface when 25 percent of the removable water remained in the blocks			Comparative rates of moisture loss in the different structural directions, when 25 percent of the removable water remained in the block		
	Longitudinal Radial	Longitudinal Tangential	Radial Tangential	Longitudinal	Radial	Tangential	Longitudinal Radial	Longitudinal Tangential	Radial Tangential
				Grams	*Grams*	*Grams*			
Red oak heartwood	2.3	3.9	1.7	0.00058	0.00038	0.00022	1.5	2.6	1.7
Bur oak heartwood	5.0	5.0	1.0	.00076	.00026	.00026	2.9	2.9	1.0
Hickory heartwood	5.0	6.7	1.3	.00050	.00022	.00017	2.3	2.9	1.3
Basswood heartwood	5.0	1.2	.2	.00760	.00099	.00466	7.7	1.6	.2
Silver maple heartwood	11.3	25.5	2.3	.00493	.00056	.00025	8.8	19.7	2.2
Silver maple sapwood	2.9	10.1	3.5	.00323	.00138	.00028	2.3	11.5	4.9
Hackberry heartwood	(2)	10.1	(2)	.00128	(2)	.00015	(2)	8.5	(2)
Hackberry sapwood	(2)	7.8	(2)	.00198	(2)	.00034	(2)	5.8	(2)
Red pine heartwood	13.0	17.2	1.3	.00378	.00037	.00027	10.2	14.0	1.4
Red pine sapwood	2.5	9.9	4.0	.00442	.00182	.00053	2.4	8.3	3.4
Average	5.9	9.7	1.9	-----	-----	-----	4.8	7.8	2.0

[1] No constant rate period or critical moisture content observed.
[2] No samples in which the moisture movement was confined to the radial direction.

A curve showing the relationship between the specific gravity and the critical moisture content for moisture movement in the longitudinal direction of the different woods is given in figure 5. Figure 6, *A* and *B*, shows the relationship between specific gravity and the rates of moisture loss when 50 and 25 percent of the total removable water remained in the wood. The curves given in figures 5 and 6 were fitted to the data by the method of least squares. It was decided that a straight line would best fit the data given in figure 5. In order to determine the type of equation which would best express the data given in figure 6, the various points for the rates of moisture loss in the longitudinal direction when 50 percent of the total removable water still remained in the wood, were plotted on logarithmic and

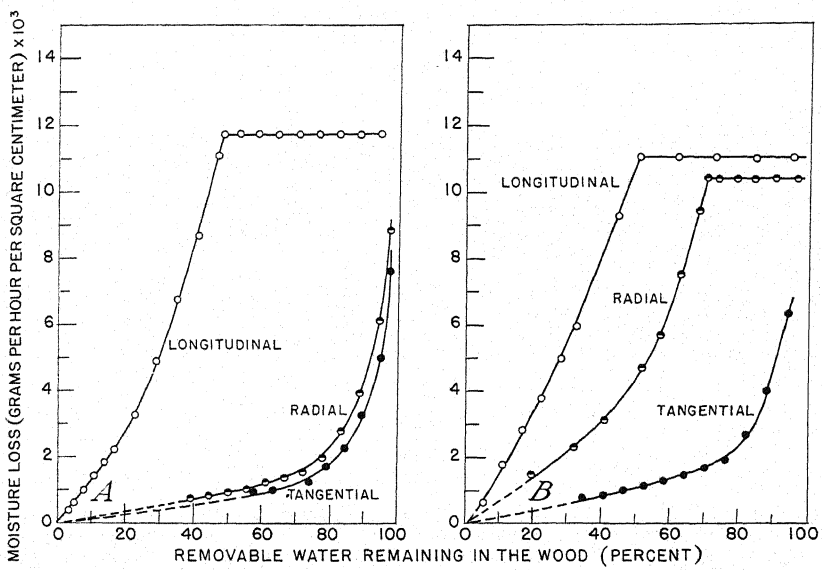


FIGURE 4.—Relation between the rate of moisture loss and the percentage of removable water remaining in the wood for the different structural directions of (*A*) red pine heartwood; (*B*) red pine sapwood.

semilogarithmic paper. A straight line was obtained on semilogarithmic paper, when the rate of moisture loss was expressed as the dependent variable and as a logarithmic function. The general equation which expresses such a relationship is

$$\log Y = a + bX$$

in which, Y is the rate of moisture loss, X is the specific gravity, a is a constant, and b is the slope of the straight line. It was assumed that the same general type equation would express the relationship between the rate of moisture loss and the specific gravity of the wood when the moisture movement was confined to the radial and tangential directions. This assumption also was used in deriving the equations for the curves showing the relationship between rate of moisture loss and specific gravity when 25 percent of the total removable water still remained in the wood.

DISCUSSION OF RESULTS

Sherwood (*18*), in some of his early papers on the drying of solids, discusses three possible mechanisms by which the water in a solid may diffuse to the surface and out into the surrounding atmosphere. These general mechanisms of drying (*18, p. 976*) are:

I.—Evaporation of the liquid at the solid surface; resistance to internal diffusion of liquid small as compared with the resistance to removal of vapor from the surface.

II.—Evaporation at the solid surface; resistance to internal diffusion of liquid great as compared with the resistance to removal of vapor from the surface.

III.—Evaporation in the interior of the solid; resistance to internal diffusion of the liquid great as compared with the total resistance to removal of vapor.

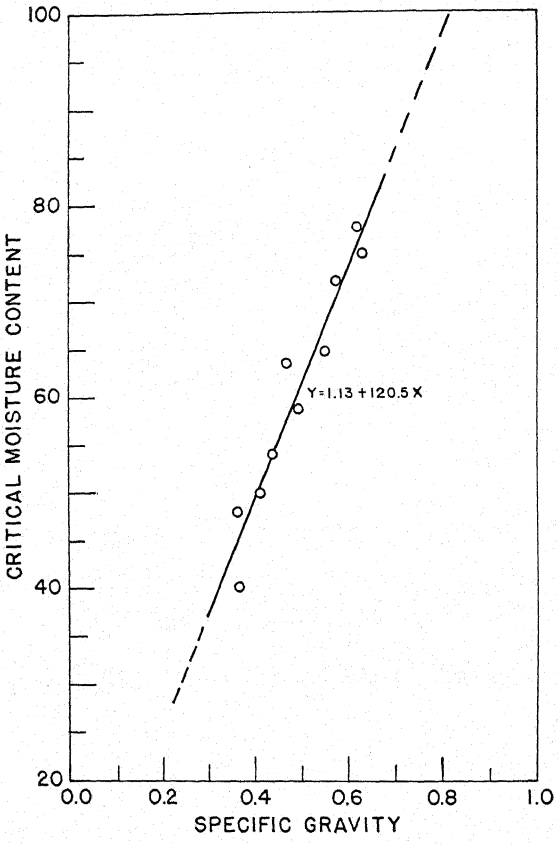


FIGURE 5.—Relation between the specific gravity and the critical moisture content of the different woods when the moisture movement was confined to the longitudinal direction.

Fisher (*5, 6*) in some of his early work on the drying of solids, concluded that the drying curves he obtained for wool, sand clay, and wood were divisible into three or four parts. Sherwood and coworkers (*3, 8, 18, 19, 20, 21*) and Luikov (*13*) have studied the drying of numerous solids from the standpoint of the foregoing theoretical considerations given by Sherwood (*18, pt. 2*) and have presented detailed mathematical analyses of the results obtained. They concluded that the drying curves for the solids used in their studies generally were divisible into three parts. Explanations were given in each case for the existence of the different portions of the drying curve. The materials investigated included wood, whiting slabs, soap slabs, compressed wood pulp, newsprint paper, brick clay, bagasse fiberboard, and peat.

Sherwood and coworkers and Luikov explain that, when the solid is very wet, the rate of moisture loss from the surface is similar to that from a free water surface; therefore, under constant drying conditions, the rate of drying remains constant. During this period, the controlling factor is the rate of evaporation through the surface air

film. As drying continues, a critical point eventually is reached on the drying curve where the rate of moisture loss begins to decrease, and the range from this point to the equilibrium moisture content is called the "falling rate period." As previously stated, the falling rate period was found to be divisible into two distinct zones called (1) the zone of unsaturated surface drying, and (2) the zone where internal liquid diffusion controls. The first of these zones follows immediately after the critical point has been reached. In this zone, it was concluded that there is a constant decrease in the rate of moisture loss with a decreasing percentage of the total removable water. The mechanism of evaporation was considered to be essentially the same as that during the constant rate period and the rate of moisture

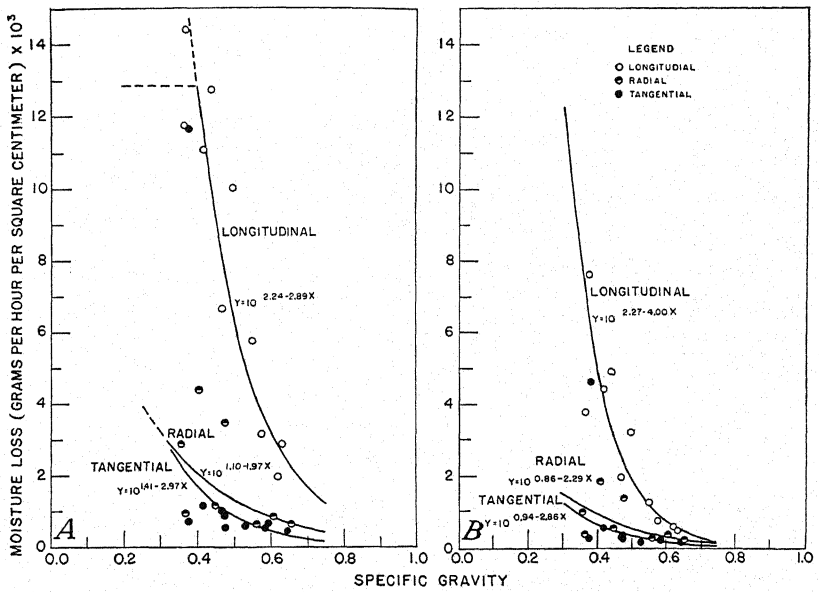


FIGURE 6.—Relation between the specific gravity and the rate of moisture loss when the moisture movement was confined to the three structural directions of the different woods and when the amount of removable water remaining in the blocks was 50 percent (*A*) and 25 percent (*B*).

loss was considered to be independent of the thickness of the material. Likewise in this zone, as during the constant rate period, the resistance to internal liquid diffusion was considered small in comparison to the resistance to vapor diffusion through the surface air film. As the rate of drying continued to decrease, a second critical point was found to exist beyond which resistance to the internal diffusion of the liquid controlled the rate of drying. During this period, it was concluded that the zone of evaporation retreated from the surface, and the water vapor was required to move through both the relatively dry surface of the solid and through the surface air film.

A study of the curves given in figures 2, 3, and 4 will show that these drying curves are divisible into a constant rate period and a falling rate period when the moisture movement was confined to the longitudinal direction of the various woods, and also when the moisture movement was confined to the radial and tangential directions

of basswood heartwood, silver maple sapwood, and to the radial direction of red pine sapwood. For the remainder of the woods the drying curves for moisture movement in the radial and tangential directions exhibited only the falling rate period. In all cases where a constant rate period is present there is evidence of an abrupt change to the falling rate period at the critical point. The results do not show, however, that the falling rate period is clearly divisible into two zones. The upper portion of the falling rate curve tends to approach a straight line, but there is no evidence of an abrupt change in the rate of moisture loss throughout this portion of the curve, thus eliminating suggestions of a second critical point. Except in the heavier woods, such as oak and hickory, most of the removable water was lost from the wood during the experiment and the drying curves approached zero, especially when the moisture movement was confined to the longitudinal direction. Considerable moisture was still present in the remainder of the blocks at the end of the experiment, but in all cases 50 percent or more of the removable water had been lost from the wood. It was noted during the latter part of the experimental run that the humidity in the cabinet had decreased slightly, thereby drying the woods at a somewhat faster rate. This had the effect of throwing the last points of the drying curve slightly out of line with the remainder, especially for those woods in which considerable moisture still was present.

A comparison of the rates of moisture loss for the different structural directions of the various woods, when 50 and 25 percent of the total removable water still remained in the wood, is given in table 2. When 50 percent of the total removable water remained in the woods, the rate of moisture loss in the longitudinal direction varied from 2.3 to 13.0 times that in the radial direction and from 1.2 to 25.5 times that in the tangential direction. The average rate of moisture loss in the longitudinal direction was 5.9 times that in the radial direction and 9.7 times that in the tangential direction. When 25 percent of the total removable water still remained in the woods, the rate of moisture loss for moisture movement in the longitudinal direction varied from 1.5 to 10.2 times that in the radial direction and from 1.6 to 19.7 times that in the tangential direction. The average rate of moisture loss, when the moisture movement was confined to the longitudinal direction, was 4.8 times the rate for movement in the radial direction and 7.8 times the rate for movement in the tangential direction. From the data in table 2, it also is evident that the rates of moisture loss for moisture movement in the radial direction varied from 0.2 to 4.0 times those for moisture movement in the tangential direction when 50 percent of the total removable water remained in the wood, and from 0.2 to 4.9 times those for movement in the tangential direction when 25 percent of the total removable water remained in the wood. The average rate of moisture loss for moisture movement in the radial direction varied from 1.9 times that in the tangential direction when 50 percent of the total removable water remained in the wood to 2.0 times that in the tangential direction when 25 percent of the removable water remained in the wood. It should be pointed out that in many cases the rates of moisture loss at the time when 25 percent of the removable water still remained in the wood were read from the extrapolated portion of the curves given

in figures 2, 3, and 4. These results show, however, that average ratios of nearly the same order of magnitude were maintained throughout the falling rate period.

The average ratios obtained for moisture movement in the different structural directions are of a similar order of magnitude to those previously reported by Buckman and Rees (*1*) for moisture movement in several coniferous woods below the fiber-saturation point. It was found that the time to attain half-saturation when the moisture movement was confined to the different structural directions was about five times as great in the radial and tangential direction as in the longitudinal direction.

The results given in table 2 show the following relative relationships between the rates of moisture loss from the heartwood and sapwood of silver maple, hackberry, and red pine when 50 percent of the total removable moisture remained in the wood. For moisture movement in the longitudinal direction of silver maple, the rate of moisture loss from the heartwood was approximately 1.3 times that for the sapwood. In the case of hackberry, the results were reversed, and the rate of moisture loss for movement in the longitudial direction of the sapwood was about 1.2 times that for the heartwood. The relative rates of moisture loss from the heartwood and sapwood of both of these woods were in the direction expected on the basis of the specific gravity relationship shown in figure 6. Both the heartwood and sapwood of red pine were still in the constant rate period when 50 percent of the removable water remained in the wood, and the variations in rate of moisture loss from the heartwood and sapwood were no greater than the normal variations for the different woods during this portion of the drying period.

Relationships comparable to the foregoing are shown by the rates of moisture loss in the heartwood and sapwood of silver maple and hackberry when 25 percent of the total removable water remained in the wood. In the red pine, however, the rate of moisture loss in the sapwood was about 1.2 times that in the heartwood, which is contrary to the more or less general relationship with specific gravity shown in figure 6.

Comparisons also can be made of the rates of moisture loss from the heartwood and sapwood when the moisture movement was confined to the radial and tangential directions. When 50 percent of the total removable water remained in the wood, the rates of moisture loss from the sapwood varied from 1.5 times that from the heartwood in the case of movement in the tangential direction of hackberry to 4.9 times that for the heartwood of red pine when the moisture movement was confined to the radial direction. When 25 percent of the removable water still remained in the wood, the rate of moisture loss from the sapwood of silver maple was 1.1 times that from the heartwood when movement was confined to the tangential direction. The rate of moisture loss in the heartwood of red pine was 4.9 times that from the sapwood when the moisture movement was confined to the radial direction. These results show that there was less resistance to moisture movement in the radial and tangential directions in the sapwood than in the heartwood.

These results do not appear to agree completely with those of Buckman and Rees (*1*), who found that the rates of moisture move-

ment below the fiber-saturation point were not significantly different in the sapwood and heartwood of Norway (red) pine and white spruce. However, in their study, comparisons could be made of the rates of moisture movement in the sapwood and heartwood of the two kinds of wood only when the moisture movement was confined to the longitudinal direction and when it was confined to the tangential direction of white spruce. As will be noted, the rates of moisture movement observed in the present study are probably not even significantly different in the heartwood and sapwood of red pine of the same specific gravities when the moisture movement was in the longitudinal direction. In addition, since these data were published, Erickson [3] has obtained information showing that there is a gradual decrease in the pressure permeability of white spruce in the longitudinal direction when samples are taken in order from the outer to the inner portions of the sapwood. Erickson, Schmitz, and Gortner (*4*) also have found that seasoning may have a significant effect on the permeability of white spruce sapwood, seasoning tending to decrease pressure permeability. This information indicates that sapwood samples selected on the basis of moisture content may tend to resemble heartwood from the standpoint of pressure permeability and also that seasoning may increase the pressure permeability of white spruce sapwood. Such data help, therefore, to explain the lack of complete agreement which may exist between the results of the two investigations insofar as these results should be correlated with the pressure permeability of the woods.

It is of considerable interest to review the data obtained in terms of the possible mechanisms of moisture movement in wood which is above the fiber-saturation point, at least at the beginning of the drying period. As previously noted, moisture may move to the surface under such conditions as free water, as bound liquid, as vapor, or by a combination of any or all of these three possible mechanisms. In all probability, all of the different mechanisms are in operation to some extent during the drying of wood above the fiber-saturation point. The primary question deals with the selection of the mechanism or combinations of such which predominate during the drying process. Certain of the pertinent factors influencing moisture movement by the different mechanisms from the standpoint of the discussion of the data given in this paper are as follows: (1) Free water movement would depend upon the size, number, and length of the capillaries between the cavities containing free water and the proportions of air and water in these cavities. Other conditions being equal, the rates of free water movement through the openings between the cavities would vary directly as the fourth powers of the radii of these capillaries. (2) Moisture movement as bound liquid would depend upon the amount of cell-wall substance along which movement can take place and the effectiveness of the cell-wall substance as a moisture-conducting medium. (3) Moisture movement in the form of vapor would depend upon the number and size of the capillary openings between the cells of the wood and the number and size of other openings that may contribute to the continuous effective open area available for vapor movement in the different structural directions, such as the presence of open vessels which furnish channels for vapor move-

[3] ERICKSON, H. D. PERMEABILITY OF WOODS TO DIFFERENT LIQUIDS AND FACTORS AFFECTING THE RATE OF FLOW. Master's thesis, Univ. of Minn. Library, Minneapolis, Minn. 1936.

ment in the longitudinal direction of the hardwoods or resin canals which may contribute to the continuous effective open area available for vapor movement in the longitudinal and radial directions of certain coniferous woods. For constant conditions with respect to other factors, the rate of vapor movement would vary directly as the second power of the radii of these openings.

Because of the anisotropic character of wood it seems desirable to consider moisture movement in the longitudinal direction more or less separate from movement in the radial and tangential directions. From figure 5, it is evident that there is a fairly definite linear relationship between the specific gravities of the various woods and the critical moisture contents for moisture movement in the longitudinal direction. By extending the straight line beyond the limits of the data, it is found to intersect the 100-percent line at a specific gravity of about 0.82. This indicates that the drying curve for a wood having a specific gravity of 0.82 or above, based on the green volume, would exhibit no constant rate period but rather would be comprised of only a falling rate period, at least when dried under the conditions employed in these experiments. When extended in the opposite direction, this straight line should pass through zero if the evaporation during the constant rate period is equivalent in character to evaporation from a free water surface; i. e., there should be no critical moisture content for evaporation from a free water surface. Extension of the curve on the basis of the equation derived by the method of least squares shows that it intersects the Y axis at +1.13 percent. Although this is in reasonable agreement with the theoretical result expected, based on the assumption that the rate of evaporation from the exposed surfaces of the blocks during the constant rate period is equal to the evaporation from a free water surface, there may be some significance to the slight deviation, as will be pointed out later.

The rate of evaporation during the constant rate period occurring in the drying of various solids quite generally has been assumed to be similar to the rate of evaporation from a free water surface. However, so far as the authors know, no attempt has been made to establish the magnitude of the difference in the rate of evaporation from a free water surface and the surface of a solid during the constant rate period. This has probably been due, in part, to the fact that data of sufficient reliability have not been available for the calculation of the rate of evaporation from a free water surface under the conditions employed in the drying experiments. As shown in table 2, the rate of moisture loss during the constant rate periods was of the same order of magnitude for moisture movement in the longitudinal direction of the different woods as well as in the heartwood and sapwood of the same kind of wood. The rate ranged from 0.01110 g per hour per square centimeter for red pine sapwood to 0.01440 g per hour per square centimeter for basswood heartwood, with an average rate for all of the woods of 0.01285 g per hour per square centimeter. The data of Lurie and Michailoff (*14*) furnish a basis for a calculation of values to use in comparing these results with the rate of evaporation from a free water surface under comparable drying conditions. Because certain of the constants in the general equation given by these authors must of necessity incorporate and reflect to some degree the errors which existed in a number of relationships determined for a wide

range of drying conditions, it seemed desirable to employ a portion of their basic data and derive a new equation for drying conditions of a somewhat more limited scope. Since the air velocity of 1.1 meters per second employed in the experiments reported in this paper is the same as the air velocity used in the collection of the data for curve No. 1 of figure 9 in the paper by Lurie and Michailoff, it was easily possible to calculate a new equation for drying data of more limited scope than that utilized as the basis for their general equation. The rate of evaporation and the drying potential were determined for each plotted point used as the basis for curve No. 1 in figure 9 of their paper with the aid of a microscope and an eyepiece micrometer. The following linear equation then was derived by the method of least squares from these data:

$$Y=0.1376+0.03447\ X$$

in which, Y is the $\left(\frac{W}{ZF}\right)$ of Lurie and Michailoff or the rate of evaporation in kilograms per square meter per hour, and X is their $H-h$ or the drying potential in millimeters of mercury. Inserting into this equation the value for the drying potential calculated from Recknagel's formula as given by Lurie and Michailoff, a value of 0.01868 g per square centimeter per hour is obtained for the rate of evaporation from a free water surface under the conditions employed in the experiments. In the consideration of this result as the true rate of evaporation from a free water surface for the drying conditions employed, thought should be given to the fact that the straight line fitted to the data of Lurie and Michailoff by the method of least squares does not pass through zero as it should if the data are exactly correct and the relationship is linear throughout. However, the authors believe that the equation derived by the method of least squares from the data furnishes the more reliable basis for making an approximation of the rate of evaporation from a free water surface for the drying conditions employed in their experiments.

Obviously, the rate of evaporation obtained from the foregoing calculations is higher than 0.01285 g per hour per square centimeter, which was the average rate of evaporation observed during the constant rate period for moisture movement in the longitudinal direction of all of the woods included in the study. Furthermore, it is higher than the maximum rate of 0.01440 g per hour per square centimeter obtained during the constant rate period for moisture movement in the longitudinal direction of basswood heartwood. These results indicate that the rates of evaporation during the constant rate periods were lower than the rate of evaporation from a free water surface subjected to the same drying conditions. A similar indication, although slight, is furnished by the results for the relationship between critical moisture contents and specific gravities of the different woods; i. e., the straight line fitted to the data by the method of least squares does not pass through zero as it should if the evaporation during the constant rate period is equivalent in character to evaporation from a free water surface and if the relationship between the critical moisture content and specific gravity can be correctly assumed to be linear throughout its range.

It seems that a purely theoretical consideration of the problem also supports the contention that the rate of evaporation from wood

during the constant rate period should be less than the rate of evaporation from a free water surface subjected to the same drying conditions, although it need not be greatly less. A free water level would not be maintained at the surface of the wood throughout the constant rate period. The free water would evaporate from the open ends of the cut-off fibers, tracheids, or vessels, and meniscuses would be formed across the pit membrane pores and possibly across the openings in the perforation plates between vessel segments. These meniscuses might be maintained close to the surface during the constant rate period, but such an assumption hardly seems tenable when it is considered that 60 percent of the total removable water was lost from basswood and about 25 percent of it from hickory and bur oak during this period. In any event, the process of evaporation from the exposed water surfaces of the meniscuses would be appreciably retarded as a result of the decreased amounts of surface and the decreased vapor pressure at the water surfaces across small capillaries, particularly those across the pores in the pit membranes. The rate of evaporation from these surfaces could not be equal to the rate of evaporation from a free water surface having the same area as the exposed surface of the blocks. Under these conditions, water would undoubtedly move to the surface from the free water level either as bound liquid or as vapor or by a combination of these two mechanisms. Such movement could not take place, however, unless there was a moisture gradient set up in the wood and the surface layers were reduced to a moisture content less than the fiber-saturation point. Thus, although a constant rate of evaporation may be maintained during the evaporation of a large proportion of the total removable water, the rate of evaporation from the surface of the wood must be less than the rate of evaporation from a free water surface throughout all of the drying period with the possible exception of the rate of evaporation of a very small amount of water at the beginning of this period. The rate of evaporation decreases from the constant rate maintained during the loss of varying amounts of the total removable water from the different woods as soon as the rate of moisture movement to the surface no longer equals the rate of moisture loss during the constant rate period. The surface of the wood probably is maintained close to the fiber-saturation point during the constant rate period. The data shown in figures 2, 3, and 4 furnish evidence of a sudden increase in the resistance to moisture movement to the surface of the blocks at the termination of the constant rate period and the rate of moisture loss decreased very rapidly. It seems probable that this was due to the rapid development of a relatively dry surface layer of wood through which moisture must move prior to evaporation, the surface probably being reduced rapidly to a moisture content approaching equilibrium with the temperature and relative humidity conditions maintained in the drying cabinet. As drying continued, the dry surface layer became thicker so that the rate of moisture loss gradually decreased throughout the remainder of the drying period.

Although it must be recognized that some free water movement may have taken place during the constant rate period, the data obtained in this study support the view that the movement of moisture as free water was not of predominant importance for moisture

movement in the longitudinal direction during either the constant or the falling rate periods. The most important evidence obtained in support of this conclusion is furnished by the existence of the following: (1) The relationship between specific gravity and the critical moisture content. (2) The relationship between specific gravity and the rates of moisture loss when 50 and 25 percent of the total removable water remained in the wood. (3) The relatively rapid rates of moisture loss for movement in the radial and tangential directions in comparison with the rates of moisture loss for movement in the longitudinal direction when 50 and 25 percent of the total removable water remained in the wood.

The results of a number of studies made of the pressure permeability of the sapwood and heartwood of different kinds of wood have shown clearly that no universal correlation exists between pressure permeability and specific gravity for wood systems having such diverse characteristics. A consideration of the previously mentioned factors which influence free water movement shows that a correlation of this nature should be in evidence before one can reconcile the simultaneous existence of the first two relationships noted in the preceding paragraph, for movement of free water in the longitudinal direction to be of predominant or even appreciable importance.

Assuming there is a potential force of a comparable order of magnitude tending to cause intercellular free water movement in the different structural directions, the intercellular movement of water in this form should be much more rapid in the longitudinal direction than in the radial and tangential directions. The resistance to the intercellular movement of free water for a certain distance in the radial and tangential directions would be much greater than the resistance to movement for the same distance in the longitudinal direction because of the many more pit membranes in series. In addition, free water movement in the longitudinal direction of the hardwoods should tend to take place through longitudinal vessel systems. The resistance to intercellular movement through the pit membranes alone should be of the order of magnitude of 50 to 100 times greater for movement in the radial and tangential directions, than for movement in the longitudinal direction of woods such as those included in the investigation. On this basis alone, irrespective of the influence which the vessels might have on free water movement in the longitudinal directions of the hardwoods, the data given in table 2 show that the rates of moisture loss for movement in the radial and tangential directions as compared to movement in the longitudinal direction are much greater than those which would be expected if moisture movement in the form of free water was of appreciable importance. Apparently, the forces tending to cause free water movement in the longitudinal direction do not have the same opportunity to exert themselves as they do when the moisture moves in the radial and tangential directions, or the movement of moisture in the form of free water is not of appreciable importance for moisture movement in any of the different structural directions. More will be said about the possible importance of free water movement in the radial and tangential directions later in the discussion.

With the elimination of the possibility of free water movement as an important factor influencing the rate of moisture movement in the

longitudinal direction, there are, as previously mentioned, three other ways in which moisture can move from the interior of the wood to the surface, namely, as vapor, as bound liquid, or by a combination of vapor movement across the cell cavities and bound liquid movement through the cell walls. The presence of the rather definite relationships between the rates of moisture loss and specific gravity for the different groups of blocks comprised of heartwood and sapwood of different kinds of wood essentially eliminates the possibility of moisture movement from the interior of the wood to the surface predominantly in the form of vapor. It would be necessary to assume that the total effective cross-sectional area available for vapor diffusion bore the same relationship to specific gravity as the rates of moisture loss if one were to attempt an explanation of the observed relationships in terms of moisture movement from the interior of the wood to the surface predominantly in the form of vapor.

As was previously pointed out (*1*), Johnston and Maass (*12*) have found that the rate of pressure movement of water through Norway (red) pine sapwood was more than 200 times that for heartwood from the same tree. These results, of course, do not apply directly to movement of vapor through wood since the rate of vapor movement would vary as the second power of the radii of the effective openings while the pressure movement of water through wood varies as the fourth power of the radii of these openings. They do show, however, that there was a decrease in either the number or size of the openings present, or both. A decrease in either the number or size of the openings would result in a decreased rate of vapor movement from the interior of the wood to the surface. It is possible, although not probable, that the sapwood and heartwood of red pine could exhibit such decidedly different permeabilities from the standpoint of the pressure movement of water and at the same time be of essentially the same permeability from the standpoint of vapor diffusion. In order for this to be true, it is necessary to assume that there is an appreciable decrease in the size of the openings which would influence the pressure movement of water to a greater extent than it would vapor diffusion, and that there is a compensating increase, from the standpoint of vapor diffusion, in the number of openings during the change from sapwood to heartwood. Such assumptions do not seem tenable, particularly in view of the work of Griffin (*9*, *10*) and Scarth (*17*) which show that the more probable thing to expect is a decrease in the number of openings during the change from sapwood to heartwood as a result of an increase in the number of aspirated bordered pits. A decrease in the number of aspirated pits would influence the rate of pressure movement of water and vapor diffusion to the same relative extent. The decrease in number of openings, of course, also may be accompanied by a simultaneous decrease in effective size of the openings, this change influencing the two types of movement in the same direction but to the different degrees noted.

Extension of a general thought somewhat comparable to that presented in the comparison of red pine sapwood and heartwood to the hardwoods having different structural characteristics also yields evidence against the possibility of moisture movement in the longitudinal direction from the interior of the wood to the surface predominantly in the form of vapor. Bur oak heartwood is very impermeable to the movement of liquids and gases because the springwood vessels of this

wood are completely plugged with tyloses. The springwood vessels of red oak heartwood, on the other hand, do not possess tyloses and they can be readily penetrated by liquids and gases. Nevertheless, the rates of moisture loss for movement in the longitudinal direction, when both 50 and 25 percent of the total removable water remained in the wood, were less than those for bur oak, the difference in rate being in the direction expected on the basis of the relationships between rate of moisture loss and specific gravity shown in figure 6. When we compare the rates of moisture loss for moisture movement in the longitudinal direction of basswood and red pine heartwood, two woods having essentially the same specific gravity, we note differences in the rates of moisture loss in the direction expected on the basis of the relative opportunities for vapor movement from the interior of the wood to the surface. However, these differences are not of the magnitude which could be justly expected if moisture moved from the interior of the wood to the surface predominantly in the form of vapor. Stamm (*22*) has determined the continuous effective open areas for transverse sections of Sitka spruce, Alaska cedar, western red cedar, Douglas fir, and western yellow pine and found that they varied from 0.33 to 1.30 percent of the total membrane area tested, with an average value for the five woods of 0.66 percent. Although red pine heartwood was not included in this group, it seems reasonable to assume, when some consideration is given to results obtained by Erickson, Schmitz, and Gortner (*4*), that the continuous effective open area would be of somewhat the same general order of magnitude for this wood. French, as quoted by Forsaith (*7, footnote, p. 42*), has observed that the pores constitute approximately 55 percent of the cross section of basswood. The continuous effective open area will probably be less than this value because of some constriction at the point of coalescence of the vessel members. However, the vessel perforations of basswood are simple and the continuous effective open area would not be greatly less than the value observed by French since at least a small portion of the decrease in the continuous open area due to constrictions in the vessels would be compensated for by the contribution of the intercellular openings between the fibers to the total continuous effective open area. Consequently, although there is evidence of some moisture movement from the interior of basswood to the surface in the form of vapor, the results support the contention that the difference in the rates of moisture loss from basswood and red pine heartwood should have been much greater than that observed if moisture movement in the longitudinal direction is predominantly in the form of vapor.

The recent work of Stamm (*24*) furnishes data in confirmation of the findings of Johnston and Maass (*12*) and Buckman, Schmitz, and Gortner (*2*). Using a somewhat different method, Stamm likewise obtained evidence of a decrease in the effective size of the intercellular openings with increasing moisture content of the wood below the fiber-saturation point. In view of such information, one cannot account for the sudden decrease in the rate of moisture loss which was observed in the present study after the critical point had been reached in terms of the influence of moisture content on the size of the openings through which vapor movement can take place. The movement of moisture predominantly in the form of vapor would have

been facilitated rather than retarded by a rapid drying of the surface layers of the wood insofar as the movement through the intercellular openings is concerned, while in the case of movement through the vessels of the hardwoods, the results of another research of Stamm (*25*) show that there would have been little, if any, influence in either direction. Martley (*15*) has noted, however, that there is an appreciable decrease in the rate of moisture movement through wood with decreasing moisture content of the wood below the fiber-saturation point. It seems that this decrease must result from an appreciably increased resistance to moisture movement through the cell walls in the form of bound liquid which probably is caused by a decrease in the size of the capillaries between the structural units of the cell wall. If such is the case, a qualitative explanation is furnished for the sudden decrease in rate of moisture movement which figures 2, 3, and 4 show occurred with relatively little change in the moisture content of the entire block. Martley's results show that a sudden reduction in the moisture content of the surface layer of the block to approximate equilibrium with the relative humidity employed in the experiments would have created an appreciably increased resistance to moisture movement to the surface and would have caused a sudden change in the rate of moisture loss comparable in general nature to that observed.

Consideration of the data for moisture movement in the longitudinal direction with regard to possible evidence favoring the movement of water from the interior to the surface predominantly in the form of bound liquid shows that there is little, if any, evidence in support of such a view. Again, it must be recognized that the possibility of some bound water movement from the interior to the surface during the constant rate period cannot be completely eliminated on the basis of the available data. However, the existence of the three relationships shown in figures 5 and 6 seems to essentially eliminate the possibility of bound water movement in appreciable amounts throughout the entire drying process. Unless the data are interpreted in this manner, it is necessary to assume that the added amount of cell-wall substance present in the woods of higher specific gravity was not only totally ineffective in conducting bound water, but that it decidedly retarded the movement of moisture from the interior to the surface in the form of bound water. Despite the fact that a progressively larger proportion of the total removable water consisted of bound water in the case of the woods of higher specific gravity and the rate of moisture loss was undoubtedly reduced somewhat because of this factor, the foregoing assumptions are untenable and do not merit further consideration. This is particularly true in view of the relatively close agreement which Stamm (*23*) has observed between the fiber-saturation points of a number of different woods.

In accordance with the results of the preceding analysis, it seems that moisture must move in the longitudinal direction of wood predominantly by means of vapor movement across the cell cavities and bound liquid movement through the cell walls. Moisture movement from the interior of the wood to the surface in such a manner is in agreement with the relationships shown in figures 5 and 6, provided it can be correctly assumed that the rate of vapor movement across the cell cavities is appreciably faster than the rate of bound liquid

movement through the cell walls. The results of the calculations previously made by Buckman and Rees (*1*) show that the rates of vapor movement across the cell cavities and bound liquid movement through the cell walls were approximately in the ratio of 100 to 1 for the conditions of their experiment. In view of the results obtained by Martley (*15*), the relative rates of moisture movement through the cell wall would have been faster for the experiments reported in this paper than for those previously conducted by Buckman and Rees because of the higher moisture content of the cell-wall substance throughout the course of the later experiments. Study of all of the information available shows, however, that there still would be a pronounced difference in the relative rate of vapor movement across the cell cavities as compared to the rate of movement through the cell walls, the relative rates being of the order of magnitude of at least 30 to 1. The existence of differences between the rates of moisture movement in the two forms provide a semiquantitative explanation for the relationships shown in figures 5 and 6. With increasing specific gravity of the wood there will be a lengthening of the relative distance that moisture must move in the form of bound liquid, a shortening of the relative distance in which moisture must move in the form of vapor, and a decrease in the relative cross-sectional area of the cell cavities through which vapor diffusion can take place.

Moisture movement in the radial and tangential directions seems to be the result of the operation of a larger number of factors, at least in some instances, than in the case of moisture movement in the longitudinal direction. The data presented in figure 6 show deviations from a general relationship between specific gravity and the rates of moisture movement in the radial and tangential directions when 50 and 25 percent of the total removable water remained in the wood. By far the most outstanding deviation from the general relationship was observed for moisture movement in the tangential direction of basswood heartwood. The rate of moisture movement in the tangential direction of basswood heartwood was very rapid, the rates of movement in this structural direction being 90 and 75 percent respectively of the rate in the longitudinal direction when 50 and 25 percent of the total removable water remained in the wood. Although the rate of moisture movement in the tangential direction of basswood heartwood was the most outstanding deviation from the general relationship between rate of moisture movement in the radial and tangential directions and the specific gravities of the woods, it is apparent that there are definite variations in the results obtained for the sapwood and heartwood of the different kinds of wood which cannot be entirely explained by differences in specific gravity. The rates of moisture movement in the radial and tangential directions of red pine and silver maple sapwood were greater than those in the same structural direction of red pine and silver maple heartwood despite the fact that exact agreement with the rate of moisture movement-specific gravity relationship would require the reverse to be true or that no appreciable difference should exist as in the case of tangential movement in silver maple. These results combined with the available information on the permeability of sapwood and heartwood qualita-

tively indicate the existence of some degree of relationship between permeability and the rates of moisture movement in the radial and tangential directions of wood.

The foregoing indications of some degree of relationship between permeability of the wood and the rate of moisture movement in the radial and tangential directions may have been due to increased amounts of water moving from the interior of the wood to the surface either in the form of vapor or free water. Buckman and Rees (*1*) previously observed pronounced deviations from a relationship between specific gravity and rate of moisture movement in the radial and tangential directions of coniferous woods below the fiber-saturation point. Under the conditions of this experiment the possibility of free water movement was, of course, eliminated, and the deviation observed must have been due to moisture movement in the form of vapor to an increased extent in the case of a number of the woods. The data obtained in the present study do not permit one to evaluate the extent to which vapor movement from the interior of the wood to the surface contributed to the deviations from the rate of moisture movement—specific gravity relationships for moisture movement in the radial and tangential direction. The relative amount of moisture which may have moved to the surface in the form of vapor cannot be determined separately from the amount which may have moved to the surface in the form of free water. However, moisture movement in either form is dependent upon the number and size of the openings through which the movement can take place, the size of the openings influencing the two types of movement to the different degrees previously noted. Further experiments are being started to determine the relative importance of movement in the form of vapor and movement in the form of free liquid as the factors causing the deviations from the rate of moisture movement-specific gravity relationships for moisture movement in the radial and tangential directions.

SUMMARY

Blocks about 2.5 by 2.5 by 5.0 cm were prepared from six hardwoods and one softwood, including the heartwood and sapwood from the same log whenever possible. These blocks were slowly dried to equilibrium with the atmosphere of the room, in which condition they were kept until the beginning of the experiments. They then were soaked in water until completely saturated and subsequently coated with 16 coats of rubber latex in such a way as to confine the subsequent moisture movement to one structural direction of the wood. They then were exposed to a temperature of 30° C. and a relative humidity of 81 percent and were weighed at suitable intervals to determine the total moisture loss at the end of various periods of time. Using these data, the average moisture loss for the various sets of blocks was calculated, and curves were constructed showing the average moisture loss with time. The rates of moisture loss at various times during the drying process were determined from the slopes of tangents to moisture loss—time curves at regular intervals. The percentage of removable water remaining in the wood also was calculated at each point for which the tangent was constructed. From

these data, curves were drawn showing the rate of moisture loss versus percentage of removable water remaining in the wood.

All of the curves for the rate of moisture loss plotted against the percentage of the total removable water when movement was confined to the longitudinal direction were found to be divisible into two periods: (1) A constant rate period, and (2) a falling rate period. When the moisture movement was confined to the radial and tangential directions constant rate periods were observed for only silver maple sapwood, basswood heartwood, and in the case of radial movement, for red (Norway) pine sapwood.

The existence of the constant and falling rate periods was considered from the standpoint of the drying of wood. The rates of moisture loss during the constant rate period were of the same order of magnitude regardless of the kind of wood or the direction of flow, and information was presented supporting the view that the rate of moisture loss during the constant rate period was somewhat less than the rate of evaporation from a free water surface.

A linear relationship was found to exist between the critical moisture content (the percentage of the removable water remaining in the wood at the termination of the constant rate period) and the specific gravity of the different woods when the moisture movement was confined to the longitudinal direction.

The rates of moisture movement in the longitudinal direction varied from 1.2 to 25.5 times those in the radial and tangential directions when 50 percent of the total removable water remained in the wood. Although the differences between the rates of movement in the different structural directions were generally slightly less when 25 percent of the total removable water remained in the wood, the values were of a comparable order of magnitude.

The average rates of moisture movement in the longitudinal direction were 5.9 and 4.8 times those for moisture movement in the radial direction when 50 and 25 percent of the total removable water remained in the wood; and were 9.7 and 7.8 times those for moisture movement in the tangential direction when the same amounts of total removable water remained in the wood. The rates of moisture movement in the radial direction varied from 0.2 to 4.9 times those in the tangential direction, with respective average rates when 50 and 25 percent of the total removable water remained in the wood, of 1.9 and 2.0 times those in the tangential direction.

An exponential relationship was found to exist between the rate of moisture movement in the longitudinal direction and the specific gravity of the different woods when 50 percent of the total removable water remained in the wood. Evidence was presented that the same general type of relationship exists between the rate of moisture movement in the longitudinal direction and specific gravity when 25 percent of the total removable water remained in the wood. Likewise, the same general type of relationship seems to apply for moisture movement in the radial and tangential directions when 50 and 25 percent of the total removable water remained in the wood, although there were appreciable deviations from the general rate of moisture movement-specific gravity relationship for moisture movement in the radial and tangential directions.

The relationship between the critical moisture content and specific gravity and the relationships between rates of moisture movement and specific gravity for moisture movement in the longitudinal direction were discussed in the light of available related information and presented as information showing that moisture moves in the longitudinal direction of wood predominantly by bound liquid diffusion through the cell walls and vapor diffusion across the cell cavities.

The operation of the same general mechanism of moisture movement in the radial and tangential directions was supported by the available data. However, deviations from exact relationships between rate of moisture movement and specific gravity for moisture movement in the radial and tangential directions show that appreciable amounts of water moved from the interior of the wood to the surface either in the form of vapor or free water, or both. Evaluation of the relative importance of these two mechanisms of moisture movement was not possible from the available data, but the data showed that the opportunities for these two types of movement ultimately must be reconciled with the following: (1) The deviation from the general relationships between the rate of moisture movement and specific gravity for moisture movement in both the radial and tangential directions; (2) the lack of a general relationship between ray volume and the relative rate of moisture movement in the radial and tangential directions of different hardwoods; (3) the faster rate of moisture movement in the radial direction of red pine sapwood despite the almost complete absence of pits in the tangential walls of the tracheids; (4) a rate of moisture movement in the tangential direction of basswood which was five times the rate of movement in the radial direction, despite the relative uniform distribution of the vessels and of the pits on the radial and tangential walls of both the fibers and vessels of this wood; (5) the decreased values for the ratio between the rates of moisture movement in the radial and tangential directions in the case of silver maple and red pine sapwood as compared to the same ratios for silver maple and red pine heartwood.

LITERATURE CITED

(1) Buckman, Stanley J., and Rees, Louis W.
1935. moisture movement in coniferous wood below the fiber-saturation point. Minn. Agr. Expt. Sta. Tech. Bull. 108, 19 pp., illus.

(2) ——— Schmitz, Henry, and Gortner, Ross Aiken.
1935. a study of certain factors influencing the movement of liquids in wood. Jour. Phys. Chem. 39: 103–120, illus.

(3) Comings, E. W., amd Sherwood, T. K.
1934. the drying of solids. vii. moisture movement by capillarity in drying granular materials. Indus. and Engin. Chem. 26: 1096–1098, illus.

(4) Erickson, Harvey D., Schmitz, Henry, and Gortner, Ross Aiken.
1937. the permeability of woods to liquids and factors affecting the rate of flow. Minn. Agr. Expt. Sta. Tech. Bull. 122, 42 pp., illus.

(5) Fisher, E. A.
1923. some moisture relations of colloids.—i. a comparative study of the rates of evaporation of water from wool, sand, and clay. Roy. Soc. [London], Proc. (A) 103: 139–161, illus.

(6) FISHER, E. A.
1923. SOME MOISTURE RELATIONS OF COLLOIDS.—II. FURTHER OBSERVATIONS ON THE EVAPORATION OF WATER FROM CLAY AND WOOL. Roy. Soc. [London], Proc. (A) 103: 664–675, illus.
(7) FORSAITH, C. C.
1926. TECHNOLOGY OF NEW YORK STATE TIMBERS. N. Y. State Col. Forestry, Syracuse Univ. Tech. Pub. 18, 374 pp., illus.
(8) GILLILAND, E. R., and SHERWOOD, T. K.
1933. THE DRYING OF SOLIDS. VI. DIFFUSION EQUATIONS FOR THE PERIOD OF CONSTANT DRYING RATE. Indus. and Engin. Chem. 25: 1134–1136, illus.
(9) GRIFFIN, G. J.
1919. BORDERED PITS IN DOUGLAS FIR: A STUDY OF THE POSITION OF THE TORUS IN MOUNTAIN AND LOWLAND SPECIMENS IN RELATION TO CREOSOTE PENETRATION. Jour. Forestry 17: 813–822, illus.
(10) ———
1924. FURTHER NOTE ON THE POSITION OF THE TORI IN BORDERED PITS IN RELATION TO THE PENETRATION OF PRESERVATIVES. Jour. Forestry 22: 82–83, illus.
(11) HAWLEY, L. F.
1931. WOOD-LIQUID RELATIONS. U. S. Dept. Agr. Tech. Bull. 248, 35 pp., illus.
(12) JOHNSTON, H. WYATT, and MAASS, O.
1930. PENETRATION STUDIES: THE PATH OF LIQUID PENETRATION IN JACK PINE. Canad. Jour. Research 3: 140–173, illus.
(13) LUIKOV, A. V.
1935. THE DRYING OF PEAT. Indus. and Engin. Chem. 27: 406–409, illus.
(14) LURIE, M., and MICHAILOFF, N.
1936. EVAPORATION FROM FREE WATER SURFACE. Indus. and Engin. Chem. 28: 345–349, illus.
(15) MARTLEY, J. F.
1926. MOISTURE MOVEMENT THROUGH WOOD: THE STEADY STATE. [Gt. Brit.] Dept. Sci. and Indus. Research, Forest Prod. Research Bd. Tech. Paper 2, 22 pp., illus.
(16) PIDGEON, L. M., and MAASS, O.
1930. THE PENETRATION OF WATER VAPOR INTO WOOD. Canad. Jour Research 2: 318–326, illus.
(17) SCARTH, G. W.
1928. THE STRUCTURE OF WOOD AND ITS PENETRABILITY. Paper Trade Jour. 86 (17): 53–58, illus.
(18) SHERWOOD, T. K.
1929. THE DRYING OF SOLIDS—I–II. Indus. and Engin. Chem. 21: 12–16, 976–980, illus.
(19) ———
1930. THE DRYING OF SOLIDS—III. MECHANISM OF THE DRYING OF PULP AND PAPER. Indus. and Engin. Chem. 22: 132–136, illus.
(20) ———
1932. THE DRYING OF SOLIDS—IV. APPLICATION OF DIFFUSION EQUATIONS. Indus. and Engin. Chem. 24: 307–310, illus.
(21) ——— and COMINGS, E. W.
1933. THE DRYING OF SOLIDS—V. MECHANISM OF DRYING OF CLAYS. Indus. and Engin. Chem. 25: 311–316, illus.
(22) STAMM, ALFRED J.
1927. THE CAPILLARY STRUCTURE OF SOFTWOODS. Jour. Agr. Research 38: 23–67, illus.
(23) ———
1929. THE FIBER-SATURATION POINT OF WOOD AS OBTAINED FROM ELECTRICAL CONDUCTIVITY MEASUREMENTS. Indus. and Engin. Chem., Analyt. Ed. 1: 94–97, illus.
(24) ———
1935. THE EFFECT OF CHANGES IN THE EQUILIBRIUM RELATIVE VAPOR PRESSURE UPON THE CAPILLARY STRUCTURE OF WOOD. Physics 6: 334–342, illus.
(25) ———
1935. SHRINKING AND SWELLING OF WOOD . . . Indus. and Engin. Chem. 27: 401–406, illus.

(26) STILLWELL, S. T. C.
1926. THE MOVEMENT OF MOISTURE WITH REFERENCE TO TIMBER SEASONING. [Gt. Brit.] Dept. Sci. and Indus. Research, Forest Prod. Research Bd. Tech. Paper 1, 29 pp., illus.
(27) SUTHERLAND, J. H., JOHNSTON, H. WYATT, and MAASS, O.
1934. FURTHER INVESTIGATIONS OF THE PENETRATION OF LIQUIDS INTO WOOD. Canad. Jour. Research 10: 36–72, illus.
(28) TUTTLE, FORDYCE.
1925. A MATHEMATICAL THEORY OF THE DRYING OF WOOD. Jour. Franklin Inst. 200: 609–614, illus.

THE EFFECT OF NATURAL SELECTION IN A MIXTURE OF BARLEY VARIETIES [1]

By H. V. HARLAN, *principal agronomist*, and M. L. MARTINI, *assistant botanist*, *Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

For the past 13 years the writers have grown a mixture of barley varieties in an attempt to secure information on natural selection in barley (*Hordeum* spp.). Any analysis of changes in the population of plants in nature is beset with difficulties, for the factors that favor or discourage the best growth of species and strains are numerous and intangible. They cannot be isolated and tested one at a time under field conditions, and, since they vary from season to season, they are not readily measured except by the summary statement of census.

The problem explored in this experiment perhaps gained by its simplicity. Some of the factors that complicate the broader field of natural selection were avoided. The changes in population measured the reaction of a very limited number of pure lines of a single species, and no new forms were encountered during the test. The barley plants of the varieties used are much alike in their needs and competed for water and nutrients at the same time.

The experiment is not fully comparable to most population studies, because its original composition was wholly arbitrary. Since barley is self-fertilized, the competing types remained the same throughout the experiment. For this reason it affords an opportunity to observe the rapid elimination of types so unsuited that comparable sorts probably would not have been found in such an environment in nature. So far as the writers have been able to determine from the literature, the only similar experiment is that of Sapegin [2] with wheat, which was interrupted at the end of 4 years.

MATERIALS AND PROCEDURE

In 1923 seed of 11 varieties of barley was mixed together in such proportions that an equal number of plants of each might be expected. This mixture was sent to 10 experiment stations in the Northern and Western States to be grown in 1924. Through the kindness of men who had no direct interest in the project, the experiment was carried on for a number of years and is still being carried on at three stations.

The procedure at all points was essentially the same. The mixture was seeded at most places in field plots each year. These plots were harvested and threshed with the regular station equipment. Sufficient seed was saved to plant a plot the following year and to send a sample to Washington. These latter samples were space-planted in blocks at Aberdeen, Idaho, or Sacaton, Ariz., to determine

[1] Received for publication December 27, 1937; issued August 1938.

[2] SAPEGIN, A. A. [OBSERVATIONS ON THE CHANGES IN AN ARTIFICIAL MIXTURE OF TYPES.] Trudy Odesskoi Selsk. Khoz. Selek. Sta. Vyp. 6: 42-43. 1922. [In Russian.]

the number of plants of each of the 11 varieties in the population. At harvest time the space-planted plants were pulled and a census of 500 individuals was recorded. This number is used as the limit of population throughout the paper. The mixture consisted of Trebi, Coast, Hannchen, Manchuria, White Smyrna, Smooth Awn, Lion, Meloy, Svanhals, Gatami, and Deficiens. Nine of these varieties were easily identified, but all plants of Coast could not be distinguished readily from all plants of Trebi, so these two varieties are recorded together. This unfortunate choice of varieties obscured the trends in only three cases.

Counts were made for the maximum period of 12 years on the material from three stations. At some of the stations counts were not made for all years in which the mixture was grown. Such omissions are indicated by leaders in table 2.

EXPERIMENTAL RESULTS

The number of plants of each variety surviving in the final year at all stations is recorded in table 1. These are the end results of natural selection over a period of from 4 to 12 years. The response of a given variety to the environments afforded by the various localities is indicated by the number of plants of that variety surviving in the total population. A striking feature revealed in table 1 is the rapidity with which one or two varieties became dominant at certain places. This change was accompanied by an equally rapid elimination of other varieties. When considered in geographical sequence from east to west, omitting Arlington, Va., where winter-killing is a factor, the leading varieties are Manchuria, Hannchen, Trebi, White Smyrna, and Coast. The Trebi and Coast separation, although not made in the table, has been determined with sufficient accuracy to specify their fields of adaptation.

TABLE 1.—*Final census showing effect of natural selection in a mixture of barley varieties grown at 10 locations for 4 to 12 years, recorded as the number of plants of each of 11 varieties found in a population of 500 plants*

Variety	Number of plants of each variety in the year when last grown									
	Arlington, Va., 1928	Ithaca, N. Y., 1936	St. Paul, Minn., 1934	Fargo, N. Dak., 1930	North Platte, Nebr., 1932	Moccasin, Mont., 1936	Aberdeen, Idaho, 1936	Pullman, Wash., 1930	Moro, Oreg., 1934	Davis, Calif., 1928
Coast and Trebi	446	57	83	156	224	87	210	150	6	362
Gatami	13	9	15	20	7	58	10	1	0	1
Smooth Awn	6	52	14	23	12	25	0	5	1	0
Lion	11	3	27	14	13	37	2	3	0	8
Meloy	4	0	0	0	7	4	8	6	0	27
White Smyrna	4	0	4	17	194	241	157	276	489	65
Hannchen	4	34	305	152	13	19	90	30	4	34
Svanhals	11	2	50	80	26	8	18	23	0	2
Deficiens	0	0	0	1	3	0	2	5	0	1
Manchuria	1	343	2	37	1	21	3	1	0	0

A number of the varieties are of minor importance at all stations. Of these, Deficiens is least fitted to survive when all places are considered. Meloy is only slightly better. On the other hand, Hann-

chen, White Smyrna, and the Coast-Trebi combination are suited to a wide range of conditions. Since the Coast-Trebi totals are the sum of the number of plants of two varieties, the figures are too large to compare with those of single varieties, particularly when both Coast and Trebi are present in considerable numbers. However, the good showing of this combination at so many places is partly due to the fact that Coast is well adapted to the West, while Trebi is well adapted to the region from Idaho east. There is little Trebi left in the mixture in California and almost no Coast remaining at St. Paul and Ithaca. The environment of the stations chosen covers so wide a range that only Hannchen and the Coast-Trebi combination have survived at all of them. The elimination has been much more rapid at some places than at others. At Pullman, Wash., Arlington, Va., Moro, Oreg., and Davis, Calif., for instance, one or two varieties quickly dominated the population, whereas at Moccasin, Mont., North Platte, Nebr., and Aberdeen, Idaho, changes occurred more slowly and a greater number of varieties maintained a fair percentage of plants.

Some of the details are more apparent in table 2, where the yearly census is given for each station. In both tables there are two striking instances in which the dominant variety differs from that commonly grown by farmers in the vicinity. In New York State, Manchuria, a six-rowed sort, has become absolutely dominant, whereas most of the commercial acreage is in Alpha, a two-rowed variety similar to Hannchen. On the other hand, in Minnesota, where Manchuria and Manchuria hybrids constitute the greater part of the commercial acreage, Hannchen, a two-rowed variety, is predominant. Although two-rowed barleys are commonly grown around Ithaca, the preference for such varieties depends partly on considerations other than yield. Manchuria types produce about the same number of pounds per acre as the two-rowed, and since the seeds are smaller Manchuria is probably potentially able to produce a far greater number of plants. The behavior of Hannchen in Minnesota cannot now be fully explained. There are few, if any, fields of Hannchen grown in the northern Mississippi Valley. The variety does occur, however, as a mixture in fields, and the percentage has increased in the past 30 years. Hannchen lodges more than Manchuria and is not desired by the market, but solely from the standpoint of plant productivity it would appear that it could be successfully grown.

TABLE 2.—*Annual census of 11 barley varieties grown as a mixture at 10 stations for 4 to 12 years as determined by the identification of varieties in samples of 500 plants*

Station and variety	Number of plants and year of identification											
	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936
Aberdeen, Idaho:												
Coast and Trebi	97	104	130	136	187	159	216	214	218	176	198	210
Gatami	49	32	34	34	30	20	11	15	12	17	11	10
Smooth Awn	43	40	31	18	10	6	2	7	3	1	0	0
Lion	40	45	24	40	17	21	6	10	5	3	3	2
Meloy	37	34	26	22	8	9	10	12	7	2	2	8
White Smyrna	65	51	92	89	86	119	145	136	154	160	177	157
Hannchen	59	81	80	80	111	109	82	76	75	106	75	90
Svanhals	33	48	31	39	25	33	23	15	22	28	20	18
Deficiens	40	21	18	14	13	7	2	9	2	2	8	2
Manchuria	37	44	34	28	13	17	3	6	2	5	6	3

TABLE 2.—*Annual census of 11 barley varieties grown as a mixture at 10 stations for 4 to 12 years as determined by the identification of varieties in samples of 500 plants*—Continued

Station and variety	Number of plants and year of identification											
	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936
Ithaca, N. Y.:												
Coast and Trebi [1]	107	64	93	79	70	75	74	82	77	60	61	57
Gatami	45	34	62	53	69	46	33	40	28	31	22	9
Smooth Awn	45	43	50	43	64	47	72	51	46	51	43	52
Lion	34	27	31	34	32	44	29	30	15	19	6	3
Meloy	36	9	5	6	4	0	0	1	0	0	0	0
White Smyrna	25	16	14	7	5	1	1	0	4	1	0	0
Hannchen	75	77	75	79	55	17	11	25	38	43	62	34
Svanhals	35	52	40	47	14	8	3	6	4	5	4	2
Deficiens	43	40	12	3	1	0	0	0	0	0	0	0
Manchuria	55	138	118	149	186	262	277	265	285	290	302	343
Moccasin, Mont.:												
Coast and Trebi	105	106	93	94	83	102	110	106	81	75	96	87
Gatami	55	64	72	56	64	73	62	79	87	88	78	58
Smooth Awn	55	52	42	48	54	54	39	43	37	37	39	25
Lion	39	48	44	26	31	44	48	34	33	49	47	37
Meloy	55	35	24	29	14	12	11	14	7	5	5	4
White Smyrna	34	46	54	47	59	89	108	120	187	175	180	241
Hannchen	34	39	57	45	75	55	44	55	24	29	22	19
Svanhals	35	34	45	41	40	31	32	19	9	9	6	8
Deficiens	37	14	7	14	10	2	5	1	0	1	0	0
Manchuria	51	62	62	100	71	38	41	29	33	32	27	21
North Platte, Nebr.:												
Coast and Trebi	130	107	.....	164	139	176	229	224	.....	.....	.....	.....
Gatami	65	48	.....	49	68	46	17	7	.....	.....	.....	.....
Smooth Awn	45	38	.....	26	24	20	5	12	.....	.....	.....	.....
Lion	29	48	.....	29	18	24	27	13	.....	.....	.....	.....
Meloy	44	59	.....	34	26	11	10	7	.....	.....	.....	.....
White Smyrna	51	46	.....	73	118	149	172	194	.....	.....	.....	.....
Hannchen	44	48	.....	40	33	22	8	13	.....	.....	.....	.....
Svanhals	26	48	.....	25	38	35	21	26	.....	.....	.....	.....
Deficiens	26	10	.....	21	10	4	6	3	.....	.....	.....	.....
Manchuria	40	48	.....	39	26	13	5	1	.....	.....	.....	.....
Fargo, N. Dak.:												
Coast and Trebi	86	.....	113	113	152	156	.....	.....	.....	.....	.....	.....
Gatami	56	.....	60	40	35	20	.....	.....	.....	.....	.....	.....
Smooth Awn	50	.....	47	31	42	23	.....	.....	.....	.....	.....	.....
Lion	47	.....	38	28	9	14	.....	.....	.....	.....	.....	.....
Meloy	30	.....	9	5	2	0	.....	.....	.....	.....	.....	.....
White Smyrna	39	.....	42	46	19	17	.....	.....	.....	.....	.....	.....
Hannchen	58	.....	62	102	117	152	.....	.....	.....	.....	.....	.....
Svanhals	66	.....	75	86	77	80	.....	.....	.....	.....	.....	.....
Deficiens	13	.....	2	2	2	1	.....	.....	.....	.....	.....	.....
Manchuria	53	.....	52	47	45	37	.....	.....	.....	.....	.....	.....
Moro, Oreg.:												
Coast and Trebi	147	.....	126	.....	148	125	.....	67	24	6	.....	.....
Gatami	56	.....	81	.....	15	3	.....	0	0	0	.....	.....
Smooth Awn	59	.....	38	.....	15	10	.....	0	0	1	.....	.....
Lion	109	.....	25	.....	21	3	.....	0	0	0	.....	.....
Meloy	60	.....	35	.....	11	3	.....	0	0	0	.....	.....
White Smyrna	21	.....	57	.....	192	276	.....	394	458	489	.....	.....
Hannchen	11	.....	69	.....	60	48	.....	39	8	4	.....	.....
Svanhals	15	.....	28	.....	33	26	.....	0	0	0	.....	.....
Deficiens	11	.....	18	.....	1	0	.....	0	0	0	.....	.....
Manchuria	11	.....	23	.....	4	6	.....	0	10	0	.....	.....
St. Paul, Minn.:												
Coast and Trebi [1]	110	99	121	94	122	121	.....	99	117	83	.....	.....
Gatami	40	50	59	34	31	16	.....	5	23	15	.....	.....
Smooth Awn	61	106	65	51	55	37	.....	16	33	14	.....	.....
Lion	48	61	56	52	51	34	.....	26	19	27	.....	.....
Meloy	64	22	9	9	7	5	.....	2	2	0	.....	.....
White Smyrna	28	16	14	11	14	5	.....	3	11	4	.....	.....
Hannchen	56	59	105	149	147	215	.....	251	248	305	.....	.....
Svanhals	32	34	49	69	63	57	.....	93	38	50	.....	.....
Deficiens	14	10	1	4	1	0	.....	0	0	0	.....	.....
Manchuria	47	43	21	27	9	10	.....	5	9	2	.....	.....
Davis, Calif.:												
Coast and Trebi [2]	134	251	338	362	.....	.....	.....	.....	.....	.....	.....	.....
Gatami	29	8	4	1	.....	.....	.....	.....	.....	.....	.....	.....
Smooth Awn	19	13	7	0	.....	.....	.....	.....	.....	.....	.....	.....
Lion	34	29	14	8	.....	.....	.....	.....	.....	.....	.....	.....
Meloy	81	43	40	27	.....	.....	.....	.....	.....	.....	.....	.....
White Smyrna	46	65	33	65	.....	.....	.....	.....	.....	.....	.....	.....

[1] All or nearly all Trebi at Ithaca, St. Paul, and Arlington.
[2] All or nearly all Coast at Davis.

TABLE 2.—*Annual census of 11 barley varieties grown as a mixture at 10 stations for 4 to 12 years as determined by the identification of varieties in samples of 500 plants*—Continued

Station and variety	Number of plants and year of identification											
	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936
Davis, Calif.—Contd												
Hannchen	69	46	40	34								
Svanhals	44	24	15	2								
Deficiens	13	17	8	1								
Manchuria	31	4	1	0								
Arlington, Va.:												
Coast and Trebi [1]		253	387	446								
Gatami		62	23	13								
Smooth Awn		27	15	6								
Lion		53	19	11								
Meloy		18	6	4								
White Smyrna		28	10	4								
Hannchen		10	7	4								
Svanhals		40	20	11								
Deficiens		0	0	0								
Manchuria		9	13	1								
Pullman, Wash.:												
Coast and Trebi					159	150						
Gatami					2	1						
Smooth Awn					5	5						
Lion					10	3						
Meloy					10	6						
White Smyrna					230	276						
Hannchen					42	30						
Svanhals					28	23						
Deficiens					10	5						
Manchuria					4	1						

[1] All or nearly all Trebi at Ithaca, St. Paul, and Arlington.

DISCUSSION

Survival in competition seems to be dependent on two factors which are summaries of various unknowns. The number of plants of a given variety that will be present in any year will depend on the number (not the weight) of seeds sown and on the percentage of survival of the seedlings in competition. For theoretical purposes this may be reduced to a single mathematical factor, namely, the number of seeds produced that will produce plants the following year. This ignores some factors that are known to be present and that would affect the trends, but it does give a working basis for a calculating-machine experiment in the office. Postulating 10 varieties, the poorest of which produces 45 seeds per plant, the best, 90 seeds, and the remaining 8 separated by intervals of 5 seeds each, we would obtain the curves shown in figure 1. In this figure all populations are limited to 500 individuals.

These curves, being unhampered by effects of temperature, rainfall, and pests, are very regular. The poorest variety is eliminated promptly. The best one increases for several years at a rate that, when plotted as a curve, closely approximates a straight line. The better sorts increase as long as part of their competition is against poorer varieties. Even among those moderately poor the decrease is retarded for a year or two, while the least productive varieties are still represented by a fair proportion of plants. The second best variety does not start to decrease until one-half of the total population is made up of plants of the best variety. Eventually only one

variety would be left, but the last plants of all varieties are slow to disappear. When the population has reached the point where the best variety has 498.1 plants and the second best 1.9 plants, 10 years more are required to reduce this fraction below 1.0. Assuming that less than two plants is one plant, and less than one plant no plant, it would require 10 years to eliminate the last plant.

These theoretical curves include an obvious fallacy. It is reasonable to suppose that the number of seeds produced by the best variety would be greater in the beginning, when the poorer varieties still constituted a considerable part of the population, than later when the competition is greater. An experiment is now being devised to measure this influence, but until definite information is available it does not seem worth while to introduce the refinement of a hypothetical factor. As a matter of fact this additional factor, while undoubtedly present, is probably of minor importance. It would change the pitch of the curves, but not the type, and it is the type that is here important rather than absolute fit.

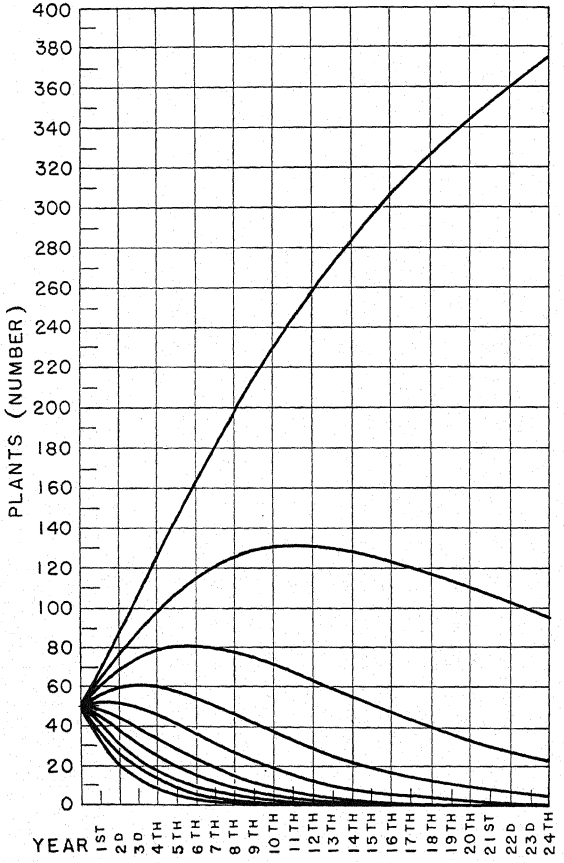


FIGURE 1.—Theoretical curves of natural selection based on an equal mixture of 10 varieties differing by 5 kernels each in their productivity per plant, the poorest plant producing 45 seeds.

The theoretical curves vary in their pitch according to the percentage difference in the intervals. The actual ones are based on many variables. In the first place, no set of 10 varieties can be chosen that are separated by equal intervals in their productivity as measured by number of seeds per plant. Also, since the climate of the United States is a continental one, the seasons favor first one variety and then another. This influence of climate may or may not be sufficient to change the order of the leading types, but in all cases it causes variations in the trend. The most exaggerated instance is Hannchen at Ithaca. At the beginning of the experiment conditions were favorable to Hannchen. Then came a series of years in which the percentage of Hannchen decreased rapidly. When the population had been

reduced from 79 to 11 plants a series of more favorable years ensued, and this variety slowly increased, reaching 62 before the onset of unfavorable conditions brought about another decline. The fluctuations of Hannchen did not, however, seriously affect the trend of the dominant variety.

It is remarkable that the curve of the Hannchen variety grown at Ithaca is the only one definitely bimodal. There are other instances of large annual fluctuations. Naturally, in the field, with widely varying seasons, uniform trends cannot be expected. At Moro, Oreg., for example, the first few years were favorable to Coast and Trebi. Then came a series of years during which Smyrna maintained an increase that charts as a nearly straight line. At Moccasin, Mont., the year 1926 was favorable for the development of Manchuria barley, and in consequence the 1927 population counted in 1928 showed a remarkable increase. The percentage of this variety declined after that time. Even in these extreme cases the fluctuations have not obscured the trends. The question is, Do the trends approach the theoretical ones? Are the actual curves of the same type as those shown in figure 1?

Three types of curves appear in figure 1, the two simple ones of the best and poorest varieties, and the humped one of the intermediate varieties. The curves of the three or four poorer varieties are very similar, differing mostly in the time required for complete elimination. It must be kept in mind that the curves are based on the number of plants rather than on percentage intervals, but nature deals with plant units. In the actual data obtained many instances of curves of rapid elimination were found. Such trends are shown by Deficiens at all 10 stations, by Meloy at Ithaca, Fargo, and St. Paul, by Smooth Awn at Aberdeen and Davis, by White Smyrna at Ithaca, and by Manchuria and Gatami at Davis. There are thus 18 or more curves of this general pattern. One of these is illustrated in figure 2, where a typical curve can be drawn through 10 of the 12 points of Meloy at Moccasin, Mont. The departures in this case seem to be due to errors of sampling rather than to seasonal fluctuations. This conclusion is based on the fact that the number of plants of this variety the following year is in line with the point where the theoretical line passed rather than on the point fixed by the census; that is, the number of plants in the population of 1929 is just what it should have been if in 1928 there had been the theoretical 18 instead of the recorded 29 plants.

Theoretically, the best variety at any place will eventually dominate the population, and the increase in numbers of the best variety is shown as a nearly straight line when plotted. The interval between the curves of the two best varieties is much greater than that between the curves of the two poorest varieties. In the counts a single variety with the characteristic dominant trend is found at most places. Typical illustrations are Hannchen at St. Paul, Manchuria at Ithaca, White Smyrna at Moccasin, Moro, and Pullman, and Coast at Davis. At Aberdeen, Fargo, and North Platte the situation is confused by the inability to separate Coast and Trebi, but with these exceptions there is no question which variety is dominant at any place. The actual data for Hannchen at St. Paul are plotted along with the theoretical curve in figure 3. The agreement is striking.

The intermediate curves shown in figure 1 may be expected to fall into two classes when applied to the actual data. Inasmuch as the seasonal fluctuations cause a degree of uncertainty in the actual trend, the five poorest varieties would not readily be separable. This is indicated in the large number already enumerated, which follow curves of rapid elimination. There then remain two curves of longer period of elimination, but more nearly approaching a straight line, and two that are definitely humped. These humped curves represent the better varieties, and the actual counts should first increase and then decrease.

The curves in figure 1 are the result of computations for 25 years. At most places a much greater interval is indicated in the actual

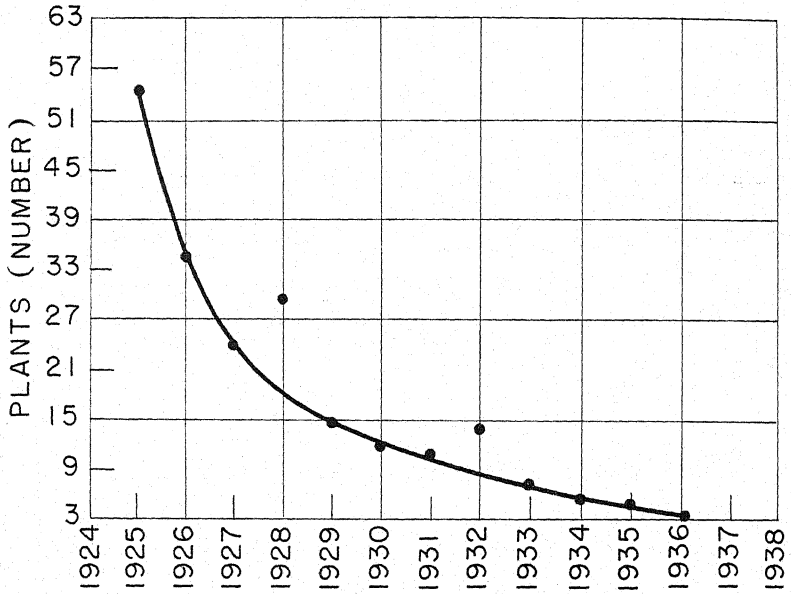


FIGURE 2.—Data on the Meloy variety at Moccasin, Mont., showing typical curve of poor variety.

data than was used in figure 1. The clearest trends in the short period of 12 years naturally came from the stations where the changes are rapid. At one or two places there are varieties in which the trend is only beginning to become definite. Definite increases in the first or later years followed by subsequent recessions are apparent in Lion at Aberdeen, Ithaca, and Moro, in Gatami at Ithaca and North Platte, in Hannchen at Moccasin and Moro, and in Smooth Awn, Manchuria, and Svanhals at Moccasin.

Data from other experiments in the field show that among varieties of nearly the same productivity the order of yield varies from year to year. The difficulty lies in determining what is fluctuation and what is trend, and how fair it may be to emphasize those curves that parallel most closely the theoretical. If carried a sufficient number of years, Smyrna and Hannchen at Aberdeen may prove to be perfect examples of nearly dominant varieties that are taking the long road to extinction. Svanhals at Aberdeen shows the trend of an average variety, with little early increase and a long period of elimination.

While not reproduced here, a good agreement of actual trend with theoretical is found if the computations are based on an interval less than that shown in figure 1. Svanhals at Moccasin follows a similar curve with a more rapid elimination. The data obtained at Moccasin are plotted in figure 4. The actual trend quite closely parallels the theoretical. In many cases good intermediate curves of greater initial increase are obtained if the data are smoothed with a moving average. Two of these, Hannchen and Manchuria at Moccasin, are shown in figures 5 and 6. In both cases the theoretical was computed with a greater interval than that used as a base in figure 1. In figure 6 it is obvious that a still greater interval would have been better. No attempt has been made in any case to find the curve that would best fit the data. This experiment is preliminary, and if the data so far obtained indicate the type of curve that may be expected, further refinements can well await the accumulation of more extensive data.

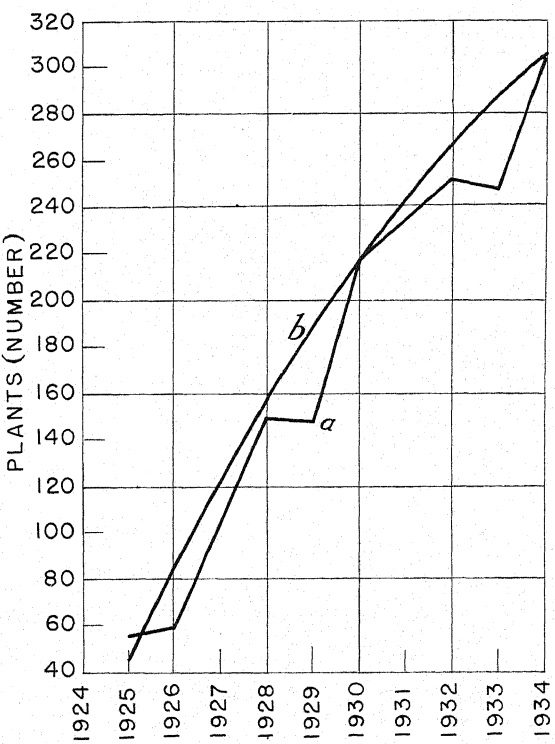


FIGURE 3.—Curve (*a*) of actual data of Hannchen at St. Paul, Minn., compared with the theoretical curve (*b*) of a dominant variety.

Naturally, many defects appear in the course of an experiment. The most important in the present work is the absence of any method of interpreting fluctuations. A new experiment has been planned that may be one step farther along, but a full understanding of all the factors that influence the results will never be achieved. A dry or a wet season may favor one variety or another. Rain on a given day may favor one variety much more than another, even though their total moisture requirements are the same. High temperatures for a few critical days may mean more than the average maximum or the absolute maximum. Some of the variables can be eliminated, others may be interpreted eventually, but many can only be accepted.

SUMMARY

A mixture of 11 varieties of barley was grown at 10 stations for a period of 4 to 12 years. The mixture was seeded, harvested, and threshed with the ordinary station equipment. Seed as it came from the thresher was saved for seeding the following year. Population counts were made annually.

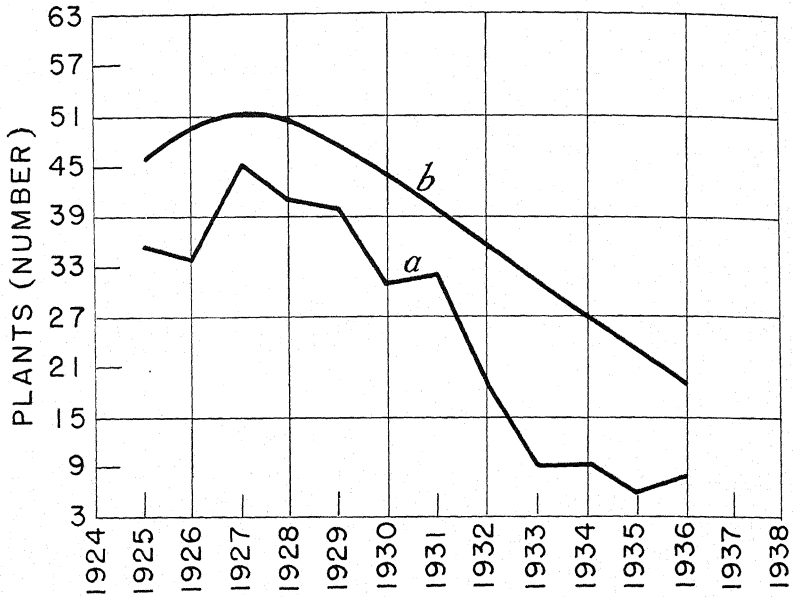


FIGURE 4.—Curve (*a*) of actual data of Svanhals at Moccasin, Mont., compared with the theoretical curve (*b*) of a variety slightly better than average.

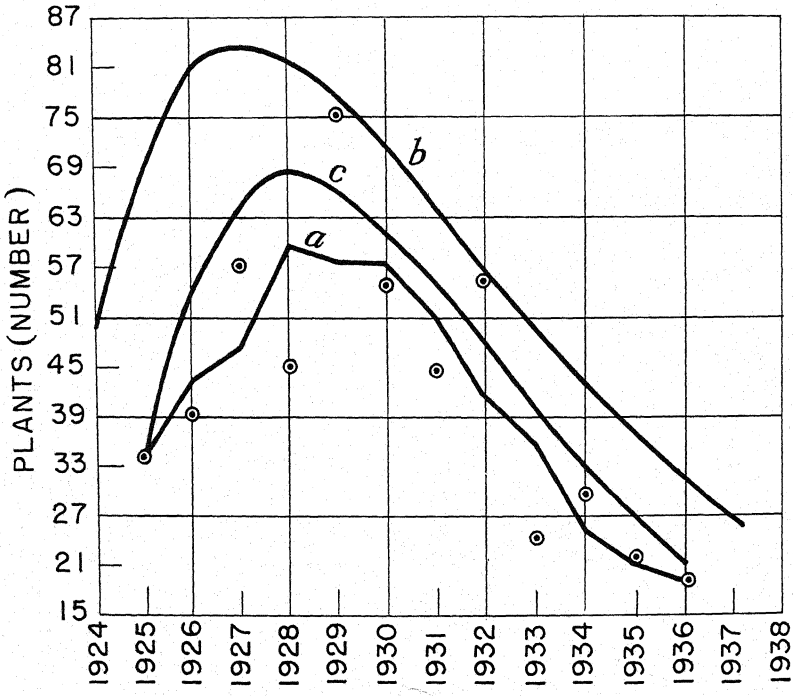


FIGURE 5.—Actual data of Hannchen at Moccasin, Mont., shown as unconnected points: *a*, The same data smoothed with a moving average; *b*, a theoretical curve as calculated for a better-than-average variety: *c*, the same curve superimposed by applying its first point to coincide with the census of 1925.

At all places there was a rapid elimination of the less adapted sorts. At most places the variety that would eventually dominate the population was quickly evident. The leading variety varied with the location of the station. A variety dominant at one station was

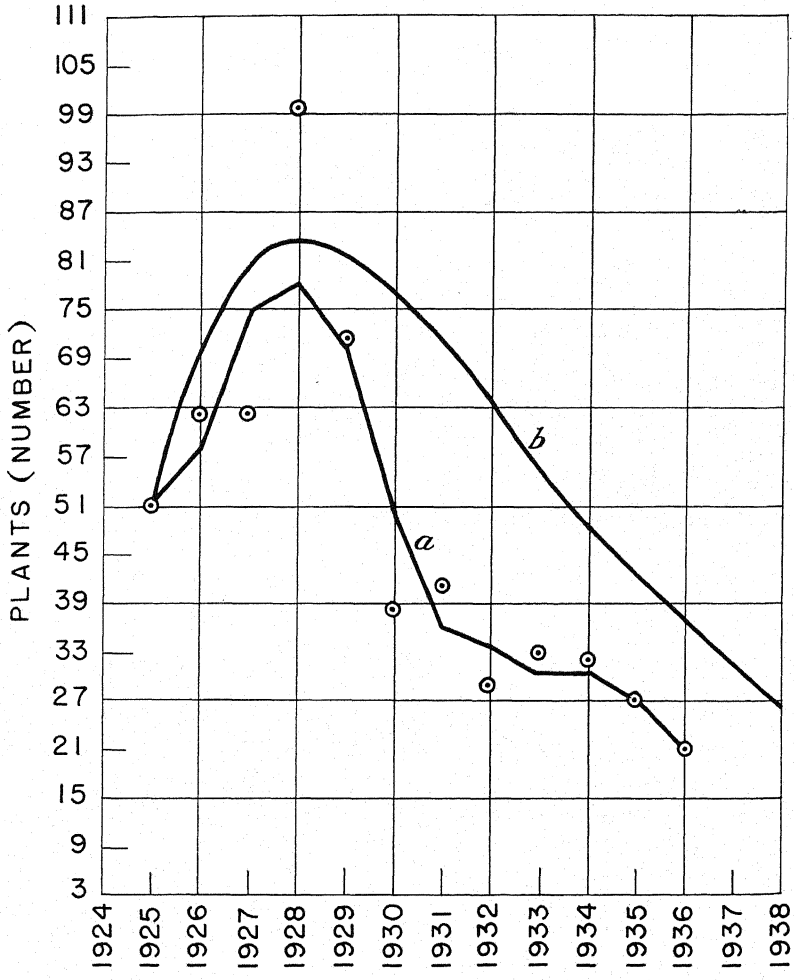


FIGURE 6.—Actual data of Manchuria at Moccasin, Mont., and the same data smoothed with a moving average (*a*) compared with a theoretical curve (*b*) of a good variety.

eliminated at another. Few varieties survived at all stations. Some varieties increased for a time and then decreased.

The population trends in general agree with a series of theoretical curves here presented. At all places the poorest varieties show the same type of descending curve. The best variety exhibits a typical ascending curve, which for a time approaches a straight line. Trends of many of the intermediate varieties are of the same type as the compound curve suggested in the theoretical scheme, first increasing, then decreasing.

EFFECT OF NEUTRAL SALTS OF SODIUM AND CALCIUM ON CARBON AND NITROGEN OF SOILS [1]

By V. P. SOKOLOFF

Formerly *junior agronomist, Division of Western Irrigation Agriculture, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Saline waters are frequently employed for irrigation purposes in the arid and semiarid regions of the Southwest. The amounts and the kinds of salts carried by irrigation waters determine to some extent the concentration and composition of the soil solution of irrigated lands. Considerable attention has been devoted, accordingly, to the study of the effects of salts and of individual ions on the physicochemical characteristics of soils. Comparatively little systematic interest has been manifested, however, in ascertaining the influence of neutral salts on the microbiological transformations of the organic matter of soils such, for example, as lead to the production of carbon dioxide and nitrates.

The conclusions of Lipman (*13*) [2], Kelley (*12*), Greaves (*11*), and Gedroiz (*8*) show a degree of accord as to the possibility of stimulating effects of small amounts of neutral salts of sodium upon nitrification in soils, at least under certain conditions. Stimulation of the nitrification processes in the presence of small amounts of neutral salts of calcium also has been reported (*13*). Higher concentrations of the neutral salts of calcium have been regarded as being more unfavorable to nitrification than corresponding concentrations of the neutral salts of sodium (*16*). The favorable effect of the oxides or carbonates of calcium on nitrification has often led to the opinion that the calcium ion, as such, is a stimulant to nitrification (*15*, *16*). Absorbed calcium, in the absence of other absorbed cations, has been shown to retard nitrification processes in the soil (*4*).

Several years ago, in an attempt to correlate the amounts of salts added to the soil, in the course of successive wetting and drying treatments, with the composition and concentration of the corresponding displaced soil solutions, the author's interest was aroused by certain fairly consistent trends in the nitrate content of displaced soil solutions. The addition of moderate amounts of sodium chloride to the soil (up to 6 milliequivalents per kilogram) was frequently followed by an increase in the nitrate concentration of the soil solution, while the addition of equivalent amounts of calcium chloride seemed to have the opposite effect. The correctness of the observations in regard to the stimulating effect of sodium chloride upon the nitrification processes of the soil was verified by simple incubation tests. At the same time the technique of the present investigation was developed. Among other things, the length of contact between soil and water during the extraction came to be regarded as an important variable,

[1] Received for publication April 25, 1938; issued August 1938. Based on a thesis submitted to the University of California in partial fulfillment of the requirements for the degree of doctor of philosophy, March 1937.

[2] Italic numbers in parentheses refer to Literature Cited, p. 215.

capable of influencing the nitrate and bicarbonate content, as well as the hydrogen-ion concentration of the extracts, especially in the presence of organic matter and of sodium salts. Periodical removal of the accumulated products of microbiological activities was likewise recognized as an important feature of the nitrification studies.

The investigation herein reported was undertaken to determine, by means of the foregoing technique, the influence of neutral salts of sodium and calcium on carbon and nitrogen transformations in two natural soils and in a third soil prepared by leaching with sodium chloride a portion of one of the original soils.

REVIEW OF LITERATURE

Lipman (*13*) observed that the nitrification processes in the soil are favored by the addition of either sodium chloride or sodium sulphate. A significant increase in the amounts of nitrate, according to Lipman, took place in the presence of 0.05 to 0.10 percent of either of these sodium salts in a soil containing 2 percent of dried blood. Higher concentrations of the salts inhibited the nitrification processes. Kelley (*12*) found that the addition of sodium sulphate to the soil stimulated nitrification under certain conditions. For example, 0.1 percent of sodium sulphate favored the nitrification of 1 percent of dried blood in a manured plot, while the same amount of the salt had no effect on the nitrification of 0.1 percent of dried blood in a control plot. The addition of ammonium sulphate to the manured plot containing 0.1 percent of sodium sulphate had no effect on the nitrate production. In the control plot, on the other hand, the nitrification of ammonium sulphate was retarded by sodium sulphate. The results of Greaves et al. (*11*) confirmed the observations of Lipman (*13*) in regard to the stimulating effect of sodium chloride on the nitrification of dried blood. Greaves and his associates, however, observed no stimulation in the presence of sodium sulphate, while calcium sulphate proved to be a stimulant within the entire range of the concentrations studied. The findings of these investigators suggest that the stimulation of nitrification, wherever it occurs, is due to the effect of individual salts on individual soils rather than to the specific influence of individual ions. The findings of Lipman and of Greaves et al. are not in agreement with the earlier results of Dehérain (*6*) nor with the conclusions of Nehring and Möbius (*15*) in a more recent investigation. It may be observed, however, that the generalizations of Nehring and Möbius, insofar as they deal with the depressing effect of sodium and potassium ions, are based on the study of the effect of sodium and potassium carbonates. The toxicity of relatively small quantities of sodium carbonate to the nitrifiers of the soil was recognized in all investigations previously discussed (*11, 12, 13*). The possibility of more rapid mineralization of the organic nitrogen of the soil in consequence of the addition of sodium salts to Chernozem soils initially free from sodium was indicated by Gedroiz (*9*). According to Gedroiz, the "optimum mobility" of the soil nitrogen may be attained when the soil solution becomes 0.01 to 0.1 normal with respect to sodium chloride, provided the air and water regimes of the soil are favorable for plant growth.

Chizhévsky (*4*), in his studies of the effect of absorbed cations upon the decomposition of organic matter in soils, found that the production of carbon dioxide was markedly stimulated by absorbed sodium and,

conversely, was depressed by absorbed calcium. The sodium-saturated soils exceeded all others in the density of their bacterial population. While both nitrification and ammonification were retarded in the calcium-saturated soils as compared with the untreated soil, no nitrogen data were reported by Chizhévsky in the case of the sodium-saturated soils. He believes, on the basis of his results with the iron-, hydrogen-, and calcium-saturated soils, that there is no parallelism between the accumulation of nitrates and the production of carbon dioxide. It may be argued, however, from Chizhévsky's own findings, that the hydrogen- and the calcium-saturated soils are not comparable among themselves, inasmuch as they differ radically in their microflora; the fungi were the only prominent group of micro-organisms that developed in the hydrogen-saturated soils, while the calcium- and the sodium-saturated soils, on the other hand, were found to be entirely comparable in this respect.

While investigators agree as to the probability of the stimulating effects of neutral sodium salts on nitrification, at least under certain conditions, the effect of the corresponding calcium salts seems to be a matter of some controversy. Waksman (*16*) states that the salts of calcium and magnesium are much more injurious to nitrification than the salts of sodium and potassium. Greaves (*11*) reports stimulation in the presence of very small quantities of calcium chloride. Nehring and Möbius (*15*) conclude that nitrification is stimulated by the calcium ion as such. It may be pertinent here to cite the observation of Kelley (*12*) that "almost any kind of conclusions may be drawn regarding the relative rates of nitrification * * *, provided that the incubation periods be carefully chosen." In order to overcome this fundamental weakness of all nitrification studies, Kelley recommends variations in the length of the incubation periods.

The effect of the neutral sodium salts on the production of carbon dioxide in the soils has received practically no attention. The greater solubility of the soil organic matter in the presence of sodium is, on the other hand, a recognized fact (*16*, *17*). The fundamentals of present knowledge in regard to nitrification and carbon dioxide production in soils are incorporated in textbooks (*7*, *16*, *17*), and need not be reviewed here. It may be remarked, however, that the older investigators paid no attention to the kind and quantity of salts inevitably present in the organic materials they have been adding to the soil and that they gave no consideration to the possibility that these salts, even in small quantities, might play a part in the transformations of the soil organic matter.

METHODS AND MATERIAL

EXPERIMENTAL PROCEDURE

Throughout the experiments 1-kg portions of soils were used. The soils were incubated at one and one-half times the moisture-equivalent moisture in parallel series, with and without artificial aeration, for several successive 15-day periods. Each period was preceded and followed by a 1-to-1 extraction. Conclusions as to the effects of the electrolytes on the transformations of organic matter in the incubated soils were based on the analyses of the 1-to-1 extracts and on the determinations of carbon dioxide and ammonia volatilized under aerated conditions.

One-kilogram aliquots of air-dried soils were extracted each with 1 liter of a N/20 electrolyte.[3] (One-tenth normal electrolytes were used with the third soil.) Distilled-water controls were included with every set of treatments, except with soil F-Na, as explained later. The extracts were separated from the soil by filtration through a double layer of Whatman No. 2 filter paper on Büchner filters. The extracts were returned to the filters repeatedly to insure uniformity of composition. The suction was continued until no more filtrate was obtained. The clear final filtrates, designated as the "zero time," were reserved for analysis. The extracted soils, generally retaining moisture corresponding to one and one-half times the moisture equivalent of the given soil, were passed through a 2-mm screen and packed loosely into 2-liter aeration flasks in such a manner as to occupy about 1½ liters of space per kilogram of the soil. The flasks containing the weighed moist soils were then aerated at room temperature for 10 hours a day at the rate of about 180 cc per minute. Day temperatures varied between 22° and 27° C.; during the night the temperature sometimes dropped below 20°.

After 15 days of such intermittent aeration the moist soils were weighed and extracted for the second time, following the technique previously indicated. Each experiment consisted of three successive 15-day aeration periods, totaling 45 days, and each of these periods was preceded and followed by a 1-to-1 extraction with a N/20 electrolyte. Parallel series of experiments were conducted with the soils incubated in stoppered jars. These unaerated soils were packed so as to occupy no more space than 0.9 liter per kilogram of soil. At the conclusion of the experiments the soils were dried in the air for 6 weeks before analysis.

The moist weight of the soils after the extraction afforded a basis for estimating the residual amounts of the dissolved substances that were carried over from period to period, on the assumption that the clear filtrate and the moisture retained by the soil were identical in composition as regards the dissolved substances. Changes in the moist weight of the soils during the aeration were taken into account in the extraction calculations. The extractions required from 1 to 6 hours each, and the subsequent screening and packing of the extracted soils required an additional 40 minutes. Two of the soils used in these investigations remained permeable to N/20 sodium chloride through the five successive extractions; in preliminary tests, difficulties had been encountered when more dilute solutions were tried. The permeability of soil F-Na was altered by presaturation with sodium; it was therefore necessary to use N/10 concentrations of the electrolytes in order to obtain a satisfactory rate of filtration, and the distilled-water series had to be omitted because of the imperviousness of the soil to water.

AERATION APPARATUS

The aeration apparatus consisted of the following units arranged in the following order:

(1) Air scrubbers: Two 2-foot towers with glass beads, inserted into 1-liter reservoir bottles containing 40 percent of sulphuric acid and 40 percent of sodium hydroxide, respectively.

[3] Calcium sulphate was used in the form of a suspension containing 50 milliequivalents of the salt per liter.

(2) One 3-foot water tower filled with beads, connected with the air scrubbers and, by means of a glass manifold tube, with the aeration flasks.

(3) Four aeration chambers: The moist air, free from carbon dioxide and ammonia, entered the flasks at the bottom through a wide glass tube and passed upward through the soil mass. A current of air was thus formed into which the gases released by the soil could diffuse. This means of aeration was preferred to other methods when the aeration periods were long.

(4) Carbon dioxide-absorbing towers: Each aeration flask was connected to a long-stemmed, fine-tipped 100-cc pipette, calibrated as to the delivery time, the tip of which touched the bottom of a reservoir flask containing 75 cc of N/2 sodium hydroxide. The incoming air lifted the alkali of the reservoir into the pipette. The small bubbles of air moving upward through the pipette and coming in contact with the film of alkali streaming down the narrow walls of the stem of the pipette were freed from carbon dioxide by the time they entered the next unit of the aeration chain.

(5) Ammonia-absorbing towers containing N/2 hydrochloric acid were constructed in the same way as the carbon dioxide absorbers. The outlets of these units were connected with the needle-valve manifold, and the outlet of the latter with the vacuum pump.

All connections of the aeration apparatus were fitted airtight and sealed with wax. Only the necessary minimum of rubber tubing was used, and all rubber was coated with paraffin. The rate of flow, initially measured with a calibrated flowmeter, was maintained nearly uniform throughout the experiment, by means of bubble count, at about 180 cc per minute, on each of the four absorbing chains. This adjustment was checked three times a week. The apparatus was placed in the basement, away from direct light and abrupt fluctuations in temperature.

ANALYTICAL METHODS

Analyses here reported were made in accordance with the procedures recommended by the Association of Official Agricultural Chemists (*2*), with the following exceptions: Total nitrogen was determined by a method combining the Devarda-alloy and the Gunning-Hibbard procedures (*5*); ammonium, by distillation in the presence of magnesium oxide (*1*); organic carbon, by a wet-combustion procedure;[4] and sodium, by weighing sodium uranyl zinc acetate hexahydrate (*3*).

The difference between the sums of nitrate, bicarbonate, sulphate, and chloride on the one hand, and of calcium, magnesium, potassium, sodium, and ammonium on the other, in no instance exceeded 0.5 percent of the total. In the case of the air-dried soils, the difference between the duplicate determinations of nitrogen (*2*) and carbon did not exceed 1 percent of each.

DESCRIPTION OF SOILS

The two sandy loam soils used in these studies were collected in the proximity of the Rubidoux Laboratory, Riverside, Calif. Both are transported soils, derived from the granites of Mount Rubidoux, and

[4] The writer is indebted to H. D. Chapman, of the University of California Citrus Experiment Station, and F. J. Foote, of the Limoneira Co. Laboratories, Santa Paula, Calif., for their modifications of the method.

both are quite similar in their characteristics, the only essential difference being that one has a much higher content of organic matter. One of these soils, designated "T," had received little or no fertilization for the past several years. The vegetative cover of this soil (trees) had been removed 1 year before the date of the collection of the sample, which was taken in January 1936 from the 6- to 12-inch horizon. The sample of the second soil, designated "F," was taken in March 1936 from the 12- to 24-inch horizon of an orange grove (plot F of the University of California Rubidoux fertilizer experiment), which had been manured repeatedly. The third soil, designated "F–Na," was a portion of soil F that had been leached with sodium chloride until about 0.9 of the original absorbed bases had been replaced by sodium. This sodium chloride-treated soil, F–Na, was rapidly dried under a fan, screened, and reserved for subsequent incubation tests.

The moisture equivalents of soils T, F, and F–Na were 11.0, 13.2, and 15.3, respectively; ammonium-absorbing capacities, 7.50, 7.87, and 8.02 milliequivalents per 100 g; total nitrogen, 6.4, 10.9, and 10.0 milligram atoms per 100 g; total carbon, 52, 126, and 122 mg atoms per 100 g. Soils F and T contained 5 and 15 milliequivalents of dissolved salts per kilogram of soil, chiefly bicarbonates, nitrates, sulphates, and chlorides of calcium and magnesium; while soil F–Na contained 60 milliequivalents of sodium chloride per kilogram and no other salts at zero time. The pH values of 1-to-1 aqueous extracts of soils F and T were 7.0 and 6.8 respectively, but no satisfactory determination of pH could be made for soil F–Na.

RESULTS

Tables 1 to 4 comprise data showing the results of the present investigation, obtained by following the foregoing experimental procedure. Concentrations of carbon and nitrogen in the soil extracts and in the air-dry soils are expressed as milligram atoms per kilogram. One milligram atom of carbon corresponds to 1 millimol of carbon dioxide, 1 milliequivalent of bicarbonate, or 12 mg of organic carbon. In a similar way, 1 mg atom of nitrogen corresponds to 1 milliequivalent of nitrate, 1 milliequivalent of ammonium, 1 millimol of ammonia, or 14 mg of nitrogen.

The findings reported in tables 1 to 4 represent primarily the effects of individual salts on individual soils under certain environmental conditions. They afford, nevertheless, a basis for some cautious generalizations. In table 5 the data are arranged with a view to facilitating such generalizations.

A preponderance of sodium salts in the soil moisture of incubated soils was found to be generally accompanied, with or without artificial aeration, by substantial increases in the amounts of nitrate and total nitrogen and of carbon dioxide and total carbon released by the soils in the course of several successive incubation extractions. The carbon-nitrogen ratios of the soils tended to become narrower as a consequence of the sodium treatment. This tendency, however, was obscured in the absence of aeration.

TABLE 1.—*Effect of sodium and calcium chlorides and sulphates on quantity of nitrogen and carbon released by aerated soils*

SOIL F

Electrolyte	Period of aeration[1]	Released per kilogram soil, oven-dry basis								
		Nitrogen					Carbon			
		Extracted			Volatilized ammonia	Total	Extracted		Volatilized carbon dioxide	Total
		Nitrate	Ammonium	Other forms[2]			Organic carbon	Bicarbonate		
		Milligram atoms	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*
Sodium chloride	First	6.22	0.14	0.15	0.05	6.56	19.8	4.4	13.9	38.1
	Second	2.48	0	.76	.37	3.61	1.0	3.2	26.3	30.5
	Third	.72	.47	.14	.37	1.70	5.6	2.3	22.6	30.5
Total		9.42	.61	1.05	.79	11.87	26.4	9.9	62.8	99.1
Sodium sulphate	First	5.82	.18	.48	0	6.48	15.9	3.7	26.1	45.7
	Second	3.70	0	0	0	3.70	4.8	1.6	24.0	30.4
	Third	1.35	0	.26	0	1.61	7.2	1.4	19.8	28.4
Total		10.87	.18	.74	0	11.79	27.9	6.7	69.9	104.5
Calcium chloride	First	5.39	.17	0	.11	5.67	8.3	2.6	15.4	26.3
	Second	2.43	0	.36	.28	3.07	.7	1.4	16.0	18.1
	Third	.79	0	.10	0	.89	2.2	.4	8.8	11.4
Total		8.61	.17	.46	.39	9.63	11.2	4.4	40.2	55.8
Calcium Sulphate	First	5.67	0	.24	0	5.81	1.9	2.1	25.2	29.2
	Second	1.61	0	0	0	1.61	2.7	.7	4.6	8.0
	Third	.89	0	.15	0	1.04	.5	1.1	12.0	13.6
Total		8.17	0	.39	0	8.56	5.1	3.9	41.8	50.8
Distilled water	First	5.39	.15	.53	.05	6.12	17.6	5.4	20.4	43.4
	Second	3.04	0	0	.23	3.27	.1	2.9	24.2	27.2
	Third	.42	0	.77	.41	1.80	3.5	1.0	8.3	12.8
Total		8.85	.15	1.30	.69	11.19	21.2	9.3	52.9	83.4

SOIL F-Na[3]

Sodium chloride	First	7.46	0.33	1.83	0	9.62	61.8	10.7	32.3	104.8
	Second	3.37	0	0	0	3.37	13.4	2.5	15.6	31.5
	Third	.62	0	.46	.02	1.10	2.5	.9	13.7	17.1
Total		11.45	.33	2.29	.02	14.09	77.7	14.1	61.6	153.4
Sodium sulphate	First	3.56	.43	3.79	0	7.78	57.7	11.1	33.0	101.8
	Second	3.16	0	0	.02	3.18	16.1	2.3	15.9	34.3
	Third	1.16	0	.63	.03	1.82	0	1.2	14.5	15.7
Total		7.88	.43	4.42	.05	12.78	73.8	14.6	63.4	151.8
Calcium chloride	First	5.13	.33	.58	.02	6.06	11.5	2.9	27.6	42.0
	Second	.74	0	.42	0	1.16	0	.2	11.9	12.1
	Third	1.28	0	0	0	1.28	0	.6	8.9	9.5
Total		7.15	.33	1.00	.02	8.50	11.5	3.7	48.4	63.6
Calcium sulphate	First	5.21	.41	.94	.01	6.57	14.8	5.0	29.1	48.9
	Second	1.31	0	71	0	2.02	0	.3	14.2	14.5
	Third	.49	0	.91	0	1.40	0	.6	10.9	11.5
Total		7.01	.41	2.56	.01	9.99	14.8	5.9	54.2	74.9

[1] Each experiment comprised 3 successive 15-day aeration periods, the first, second, and third being days 1–15, 16–30, and 31–45, respectively. Each period was preceded and followed by a 1-to-1 extraction with an electrolyte. Controls were extracted with distilled water.

[2] By difference: Total N minus NO_3, minus NH_4.

[3] With this soil, control extractions with distilled water were not technically feasible.

TABLE 1.—*Effect of sodium and calcium chlorides and sulphates on quantity of nitrogen and carbon released by aerated soils*—Continued

SOIL T

Electrolyte	Period of aeration	Released per kilogram soil, oven-dry basis								
		Nitrogen					Carbon			
		Extracted			Volatilized ammonia	Total	Extracted		Volatilized carbon dioxide	Total
		Nitrate	Ammonium	Other forms			Organic carbon	Bicarbonate		
		Milligram atoms	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*
Sodium chloride	First	9.65	0	0.61	0	10.26	8.6	2.4	15.4	26.4
	Second	.71	0	.14	0	.85	7.1	1.4	20.4	28.9
	Third	.89	0	.08	0	.97	4.9	1.6	21.4	14.9
Total		11.26	0	.83	0	12.08	20.6	5.4	57.2	70.2
Sodium sulphate	First	9.74	0	.17	0	9.91	6.7	2.7	16.9	26.3
	Second	1.10	0	0	0	1.10	4.8	.8	17.4	23.0
	Third	.32	0	.28	0	.60	4.5	1.2	7.7	13.4
Total		11.16	0	.45	0	11.61	16.0	4.7	42.0	62.7
Calcium chloride	First	9.32	0	.36	0	9.68	7.2	2.0	12.8	22.0
	Second	.64	0	.04	0	.68	.2	1.0	18.0	19.2
	Third	.96	0	0	0	.96	1.4	1.0	12.8	15.2
Total		10.92	0	.40	0	11.32	8.8	4.0	43.6	56.4
Calcium sulphate	First	7.26	0	.30	0	7.56	3.1	2.0	15.0	20.1
	Second	.91	0	0	0	.91	1.8	.6	10.2	12.6
	Third	.53	0	.18	0	.71	.9	.9	6.8	8.6
Total		8.70	0	.48	0	9.18	5.8	3.5	32.0	41.3
Distilled water	First	9.30	0	.33	0	9.63	9.4	3.5	13.4	26.3
	Second	1.08	0	.15	0	1.23	1.3	1.5	19.6	22.4
	Third	1.34	0	.03	0	1.37	3.2	1.0	13.8	18.0
Total		11.72	0	.51	0	12.23	13.9	6.0	46.8	66.7

Comparisons made in table 5 suggest the term "stimulation" as descriptive of the effect of sodium salts on the transformations of carbon and nitrogen in the soils under the experimental conditions previously outlined. The stimulating effect of the sodium salts is manifested in the increased quantities of nitrate, total nitrogen, carbon dioxide, and the soluble forms of carbon that were released in the course of the incubation-extraction treatments (tables 1 and 2). While the differences between the sodium-treated soils and the distilled-water series were not so great as the differences between the sodium-treated and the calcium-treated soils, they were significant in three cases out of four. There was no appreciable difference between the effects of sodium chloride and of sodium sulphate on the transformations of the organic matter in the soil, despite one rather striking exception (table 2). The differences between calcium chloride and calcium sulphate, however, were conspicuous and irregular. In contrast with the observations of Greaves et al. (*11*), calcium sulphate was found to be more often a deterrent than a stimulant to nitrification. The stimulating effects of the sodium salts previously shown were emphasized in consequence of the presaturation of the soil with sodium (soil F–Na). The striking increase

in the production of nitrate parallels, in some respects, the experience of Greaves (*10*) with leached alkali soils. The failure of the ionic antagonism to manifest itself in the sulphate-treated soil F–Na is rather surprising and cannot be harmonized with the results of Lipman (*14*).

TABLE 2.—*Effect of sodium and calcium chlorides and sulphates on the quantity of nitrogen and carbon released by soils in the absence of aeration*

Electrolyte	Period of incubation[1]	Released per kilogram of soil, oven-dry basis						
		Nitrogen				Carbon		
		Extracted			Total	Extracted		Total
		Nitrate	Ammonium	Other forms[2]		Organic carbon	Bicarbonate	
		Milligram atoms	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*
SOIL F								
Sodium chloride	First	6.23	0	0.57	6.80	0	1.3	1.3
	Second	1.17	0	.89	2.06	4.0	2.1	6.1
	Third	1.36	0	.61	1.97	0	1.4	1.4
Total		8.76	0	2.07	10.83	4.0	4.8	8.8
Sodium sulphate	First	7.06	0	.12	7.18	0	2.4	2.4
	Second	0	.41	.68	1.09	7.7	9.0	16.7
	Third	2.55	0	0	2.55	0	1.6	1.6
Total		9.64	.41	.80	10.82	7.7	13.0	20.7
Calcium chloride	First	6.61	0	.14	6.75	0	1.0	1.0
	Second	.21	0	.52	.73	.3	.6	.9
	Third	1.19	0	0	1.19	0	.8	.8
Total		8.01	0	.66	8.67	.3	2.4	2.7
Calcium sulphate	First	.17	1.42	.68	2.27	0	11.1	11.1
	Second	0	.64	.93	1.57	.3	6.7	7.0
	Third	1.85	0	0	1.85	0	1.6	1.6
Total		2.02	2.06	1.61	5.69	.3	19.4	19.7
Distilled water	First	7.04	0	0	7.04	0	1.2	1.2
	Second	1.30	0	.53	1.83	.9	2.8	3.7
	Third	1.05	0	.12	1.17	0	1.1	1.1
Total		9.39	0	.65	10.04	.9	5.1	6.0
SOIL F-Na[3]								
Sodium chloride	First	4.32	0.41	1.50	6.23	32.3	3.6	35.9
	Second	1.44	.01	2.78	4.23	57.1	5.2	62.3
	Third	0	.86	.27	1.13	31.0	0	31.0
Total		5.76	1.28	4.55	11.59	120.4	8.8	129.2
Sodium sulphate	First	4.32	.83	1.22	6.37	39.9	3.6	43.5
	Second	0	.15	2.59	2.74	65.0	6.5	71.5
	Third	.20	1.12	1.02	2.34	30.3	0	30.3
Total		4.52	2.10	4.83	11.45	135.2	10.1	145.3
Calcium chloride	First	1.44	.28	.50	2.22	14.1	.8	14.9
	Second	.83	.16	.34	1.33	12.6	1.3	13.9
	Third	1.11	.49	0	1.60	0	.5	.5
Total		3.38	.93	.84	5.15	26.7	2.6	29.3
Calcium sulphate	First	3.46	.41	.98	4.85	21.8	1.7	23.5
	Second	.11	.38	.48	.97	2.5	1.6	4.1
	Third	0	.52	.11	.63	2.4	.1	2.5
Total		3.67	1.31	1.57	6.45	26.7	3.4	30.1

[1] The first, second, and third 15- or 30-day periods of incubation included days 1–30, 30–45, and 45–60 respectively for soil F; days 1–15, 16–30, 31–45 respectively for soil F–Na; and days 1–15, 16–30, and 31–60 respectively for soil T. Each period was preceded and followed by a 1-to-1 extraction with an electrolyte. Controls were extracted with distilled water.

[2] By difference: Total N minus NO_3, minus NH_4.

[3] With this soil, control extractions with distilled water were not technically feasible.

TABLE 2.—*Effect of sodium and calcium chlorides and sulphates on the quantity of nitrogen and carbon released by soils in the absence of aeration*—Continued

SOIL T

Electrolyte	Period of incubation	Released per kilogram of soil, oven-dry basis						
		Nitrogen				Carbon		
		Extracted			Total	Extracted		Total
		Nitrate	Ammonium	Other forms		Organic carbon	Bicarbonate	
		Milligram atoms	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*
Sodium chloride	First	9.58	0.06	0.25	9.89	9.1	3.2	12.3
	Second	0	.03	0	.03	6.0	2.4	8.4
	Third	2.87	0	1.13	4.0	3.7	1.7	5.4
Total		12.45	.09	1.38	13.92	18.8	7.3	26.1
Sodium sulphate	First	8.99	.07	.74	9.80	7.9	2.4	10.3
	Second	0	.01	0	.01	5.1	2.0	7.1
	Third	.29	.12	.91	1.32	7.8	4.9	12.7
Total		9.28	.20	1.65	11.13	20.8	9.3	30.1
Calcium chloride	First	7.92	.26	.48	8.66	6.1	3.9	10.0
	Second	0	.01	0	.01	4.4	.4	4.8
	Third	1.70	.17	.23	2.10	1.9	1.1	3.0
Total		9.62	.44	.71	10.77	12.4	5.4	17.8
Calcium sulphate	First	8.23	.16	.55	8.94	6.5	1.7	8.2
	Second	.35	.14	0	.49	3.0	1.4	4.4
	Third	1.76	.03	.32	2.11	3.5	1.2	4.7
Total		10.34	.33	.87	11.54	13.0	4.3	17.3
Distilled water	First	7.87	.02	.35	8.24	6.5	4.3	10.8
	Second	0	.07	0	.07	3.2	.9	4.1
	Third	2.28	0	0	2.28	4.1	1.7	5.8
Total		10.15	.09	.35	10.59	13.8	6.9	20.7

An attempt to explain the stimulating effect of the sodium salts upon nitrification and the production of carbon dioxide, and the increase in the amounts of soluble nitrogen and carbon released under the influence of the sodium salts, leads, in the present state of our knowledge, to the following considerations:

The greater solubility of the organic matter of the soil in the presence of the sodium salts is indicated by the consistently larger quantities of the organic carbon that were present in the extracts of the sodium-treated soils. An increase in the solubility of the organic matter may in itself prove to be a stimulant to the microbiological processes in the soil under favorable environmental conditions. This stimulation may be due to a simple increase in the available amounts of substances needed for the development of the soil microflora, to a possible increase in the amounts of soluble phosphate in the presence of dissolved organic matter, or to some complicated and obscure influence that may be designated as the "specific" effect of the dissolved organic matter. A remarkable increase in the density of bacterial population observed by Chizhévsky (*4*) as a consequence of the saturation of the soil with sodium can be attributed either to the effect of absorbed sodium on the environment or to the increase in the soluble organic matter or to both.

TABLE 3.—*Total nitrogen and carbon in air-dry soils at the beginning and at the end of the tests*

Soil	Electrolyte	Aerated soils								Unaerated soils							
		Nitrogen per 100 g				Carbon per 100 g				Nitrogen per 100 g				Carbon per 100 g			
		Initial total	Final total	Difference	Removed during experiment	Initial total	Final total	Difference	Removed during experiment	Initial total	Final total	Difference	Removed during experiment	Initial total	Final total	Difference	Removed during experiment
		Milligram atoms	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*
F	Sodium chloride	10.9	11.4	+0.5	1.2	136	115	−21	10	10.9	9.9	−1.0	1.1	136	105	−31	1
	Sodium sulphate		10.5	−.4	1.2		108	−28	10		9.8	−1.1	1.1		105	−31	2
	Calcium chloride		11.0	+.1	1.0		122	−14	6		10.3	−.6	.9		130	−6	1
	Calcium sulphate		10.0	−.9	.9		118	−18	5		10.3	−.6	.6		118	−18	2
	Distilled water		10.1	−.8	1.1		114	−22	8		9.3	−1.6	1.0		111	−25	1
F–Na [1]	Sodium chloride	10.1	8.1	−2.0	1.4	122	93	−29	14	10.0	8.1	−1.9	1.2	122	105	−17	13
	Sodium sulphate		7.4	−2.7	1.3		95	−27	12		8.3	−1.7	1.1		105	−17	15
	Calcium chloride		8.3	−1.8	.9		106	−16	10		8.8	−1.2	.5		112	−10	3
	Calcium sulphate		9.5	−.6	1.0		105	−17	10		8.6	−1.4	.6		110	−12	3
T	Sodium chloride	6.4	4.7	−1.7	1.2	52	46	−6	8	6.4	4.5	−1.9	1.4	52	46	−6	3
	Sodium sulphate		5.2	−1.2	1.2		47	−5	6		4.4	−2.0	1.1		50	−2	3
	Calcium chloride		4.4	−2.0	1.1		49	−3	6		4.3	−2.1	1.1		48	−4	2
	Calcium sulphate		4.5	−1.9	.9		47	−5	4		4.3	−2.1	1.1		49	−3	2
	Distilled water		4.5	−1.9	1.2		46	−6	7		4.3	−2.1	1.0		50	−2	2

[1] Extraction of this soil with distilled water was not technically feasible.

TABLE 4.—*Carbon-nitrogen ratios of air-dry soils at the beginning and at the end of the tests*

Soil	Electrolyte	Ratio of total carbon to total nitrogen in—			
		Aerated soil		Unaerated soil	
		Initial	Final	Initial	Final
		Milligram atoms	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*
F	Sodium chloride	12.5	10.1	12.5	10.6
	Sodium sulphate		10.3		10.7
	Calcium chloride		11.1		12.6
	Calcium sulphate		11.8		11.5
	Distilled water		12.2		11.9
F-Na[1]	Sodium chloride	12.2	11.5	12.2	13.0
	Sodium sulphate		12.8		12.6
	Calcium chloride		12.8		12.7
	Calcium sulphate		11.1		12.8
T	Sodium chloride	8.2	9.7	8.2	10.2
	Sodium sulphate		9.0		11.3
	Calcium chloride		11.1		11.2
	Calcium sulphate		10.5		11.3
	Distilled water		10.2		11.6

[1] Extraction of this soil with distilled water was not technically feasible.

TABLE 5.—*Total nitrogen and total carbon released by soils, expressed as percentages of the calcium chloride-treated series*

Electrolyte	Total nitrogen in—						Total carbon in—					
	Aerated soil			Unaerated soil			Aerated soil			Unaerated soil		
	F	F-Na	T	F	F-Na	T	F	F-Na	T	F	F-Na	T
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Sodium chloride	123	166	107	125	225	129	178	241	136	326	441	147
Sodium sulphate	122	150	103	125	222	103	187	239	112	767	496	169
Calcium chloride	100	100	100	100	100	100	100	100	100	100	100	100
Calcium sulphate	89	118	81	66	125	107	91	118	73	730	103	97
Distilled water	114	(1)	108	116	(1)	98	149	(1)	118	222	(1)	116

[1] Extraction of this soil with distilled water was not technically feasible.

The greater solubility of the organic matter in the sodium-treated soils cannot be understood so easily as some superficial considerations would lead us to believe. Neither the percentage of sodium in the soil extracts, nor the pH trends, nor the amounts of carbon dioxide released, nor the amounts of nitrogen nitrified can be related directly to the amounts of humus extracted at any given time. Nor is there any significant parallelism between any two of the above-enumerated quantities insofar as the rates of their release are concerned. The total amounts of carbon, however, released from period to period (1–15 days, 16–30 days, etc.) do parallel the total amounts of nitrogen released during the same period, in the sense that the greater amounts of one frequently correspond to the greater amounts of the other. The grand total amounts of nitrogen and carbon, on the other hand, representing the cumulative effects of the entire experiment, suggest the possibility of correlation, if not of proportionality. It may be observed here that the carbon-nitrogen ratio of the extracts of all

sodium-treated soils is substantially higher than it is for the corresponding calcium-treated soils.

The amounts of nitrogen released by the soils invariably decreased from period to period. The bulk of nitrate and of the total nitrogen was, as a rule, extracted after the first 15 days of the incubation. There was no such abrupt change in the production of carbon dioxide, however, while the amounts of the soluble carbon, after a drop between the fifteenth and the thirtieth day of the incubation, would often increase during the next 15-day period. The stimulating effect of the sodium salts was repeatedly manifested throughout the entire range of these fluctuations, although the degree of stimulation varied considerably from time to time and from soil to soil.

The fluctuations in the rate of the transformations of organic matter in the soils suggest, perhaps, a degree of periodicity in the activities of the different groups of the soil micro-organisms. The reactions of the carbon and nitrogen cycles in the soil, though interdependent, may not be entirely contemporaneous. Moreover, as the relative proportions of the inorganic constituents in the soil moisture are altered, in the course of the present experiment, in the direction of a complete proponderance of either sodium or calcium salts, the possibilities of the ionic antagonism (*14*) become minimized.

The release of nitrogen by the soils was stimulated to the extent of 7 to 70 percent in the presence of sodium salts if the comparison be made on the basis of the corresponding and parallel calcium-treated series. A far greater stimulation was observed in the transformations of the soil carbons. It would seem reasonable therefore that the sodium-treated soils, having lost relatively more carbon than nitrogen in the course of the experiment, would be left with a narrower C : N ratio than they had heretofore possessed. This, however, was not always the case (table 4).

There were unaccounted-for gains in nitrogen, suggestive of nitrogen fixation, in soil F. The unaccounted-for losses of both carbon and nitrogen (table 3), observed in the majority of cases, had probably taken place during the 6 weeks' drying in the air that followed the incubation-extraction treatments, and, perhaps, during the extraction periods. Significant as these losses are, they scarcely invalidate the measurements of either the released or the residual amounts. The C : N ratio of soil F tended to become narrower, regardless of treatment. This tendency, however, was much more emphatic in the presence of sodium salts than in the presence of calcium salts. Soil T, on the other hand, tended to increase its C : N ratio in all instances. Here, again, the narrower ratio was found in the case of the sodium-treated soils. It appears, accordingly, that, at least in this particular instance, sodium salts exert an influence of their own on the C : N ratio of the soil.

Among minor observations incidental to the main purpose of the experiment, the effect of sulphate on the unaerated soil F is to be noted. Here the preponderance of gypsum, as well as of sodium sulphate, though to a lesser extent, was soon accompanied by the appearance of large amounts of bicarbonate and ammonia and by the odor suggestive of the reduction of the sulphates. After 30 days nitrate disappeared from the soil solution. It is of interest that the 30-day extract of the gypsum-treated soil attains the threshold of phenol-

phthalein alkalinity. It is likewise of interest that the reducing properties of sulphate-treated soils apparently lost some of their intensity some time after the 45 days' incubation. No such effects were observed in the other two soils, although the nitrification apparently ceased in both (with the exception of the calcium chloride-treated F–Na and the calcium sulphate-treated T) after 15 to 30 days' incubation.

The release and volatilization of ammonia by the aerated soil F can hardly be harmonized either with characteristics of the medium (such as its aerobic character and only faintly alkaline pH) or with the opinion that the release of ammonia in the soils is one of the consequences of the narrowing of their C : N ratio (*17*). One may suppose that, in this particular soil, ammonia had been removed by the stream of air before it could be nitrified, on the general assumption that ammonification normally preceded nitrification.

CONCLUSIONS

Conservation of soil organic matter in the form of stable humus reserves is regarded by agronomists as an important aspect of the major problem of maintaining the productivity of soils. It has been recognized in this connection that sodium, absorbed or present in the soil solution, tends to promote depletion of organic reserves of the soil (*9*). Emphasis heretofore has been placed on sodium primarily in connection with high alkalinity. The results of the present investigation indicate that neutral salts of sodium, even in concentrations sufficient to prevent deflocculation of the soil, dissolve significant quantities of organic matter. The presence of neutral salts in soils can be regarded, accordingly, as a factor unfavorable to the conservation of organic matter.

The stimulating effect of sodium salts on mineralization of nitrogen and, perhaps, on the solubility of other nutritive substances of the soil, has agronomic consequences and invokes important nutritional considerations. To what extent the beneficial effects that sometimes have been observed to follow the application of sodium chloride to lands can be attributed to the sodium effect as distinct from the chloride effect cannot now be stated. The results here presented suggest, however, that in many instances the observed responses have occurred at the expense of the organic reserves of the soil.

SUMMARY

The effect of sulphates and chlorides of sodium and calcium on the microbiological transformations of the organic matter in two sandy loam soils was studied by determining volatile and soluble products of bacterial action that were released by aerated soils in the course of three successive 15-day incubation periods, each preceded and followed by a 1-to-1 extraction with N/20 solutions of the indicated electrolytes. Parallel series of tests were conducted with the same soils incubated in the absence of artificial aeration. The experimental results suggested the following conclusions:

(1) The release of nitrate and of total nitrogen, as well as of the soluble and volatile forms of carbon, is stimulated, often to a great

extent, by the sodium salts and depressed by the corresponding calcium salts, regardless of aeration.

(2) Presaturation of the soil with sodium tends to emphasize the stimulating effects of the sodium salts.

(3) The carbon-nitrogen ratio of the undissolved organic matter of the soil is lower in the sodium-treated than in the calcium-treated, aerated soils.

Without attempting to explain the mechanism of the stimulating effects of sodium salts, the author discusses these effects with reference to the influence of sodium on the soil organic matter and to the possible physiological influences of the soluble organic matter, as well as of the electrolytes themselves, on the activities of the organisms responsible for the carbon and nitrogen transformations in the soil.

LITERATURE CITED

(1) AMERICAN PUBLIC HEALTH ASSOCIATION.
1925. STANDARD METHODS FOR THE EXAMINATION OF WATER AND SEWAGE. Ed. 6, 119 pp., illus. New York.

(2) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.
1930. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS . . . Ed. 3, 593 pp., illus. Washington, D. C.

(3) BARBER, H. H., and KOLTHOFF, I. M.
1928. A SPECIFIC REAGENT FOR RAPID GRAVIMETRIC DETERMINATION OF SODIUM. Jour. Amer. Chem. Soc. 50: 1625–1631.

(4) CHIZHÉVSKY, M. G., and KHLUSTIKOVA, M. M.
1932–33. [INTENSITY OF THE DECOMPOSITION OF ORGANIC MATTER IN THE SOIL AS INFLUENCED BY THE KIND OF ABSORBED CATION]. Chemisation Socialist. Agr. 1932, No. 7, pp. 6–16; No. 8, pp. 25–34; 1933, No. 2, pp. 106–112. [In Russian.]

(5) DAVISSON, B. S., and PARSONS, J. T.
1919. THE DETERMINATION OF TOTAL NITROGEN INCLUDING NITRIC NITROGEN. Jour. Indus. and Engin. Chem. 11: 306–311, illus.

(6) DEHÉRAIN, P. P.
1887. SUR LA PRODUCTION DES NITRATES DANS LA TERRE ARABLE. Ann. Agron. 13: 241–261.

(7) DEMOLON, ALBERT.
1932. LA DYNAMIQUE DU SOL. 347 pp., illus. Paris.

(8) GEDROIZ, K. K.
1926. [MOBILITY OF SOIL COMPOUNDS AS INFLUENCED BY CALCIUM.] Nosovka Selsk. Khoz. Opytn. Sta., Otd. Agron. 43, 18 pp., illus. [In Russian.]

(9) ———
1928. ALKALI [SOLONETZ] SOILS, THEIR ORIGIN, PROPERTIES, AND IMPROVEMENT. A SCIENTIFIC POPULAR SKETCH. Nosovka Selsk. Khoz. Opytn. Sta., Otd. Agron. 46, 73 pp. [In Russian. Title also in English.]

(10) GREAVES, J. E.
1927. THE MICROFLORA AND PRODUCTIVITY OF LEACHED AND NONLEACHED ALKALI SOILS. Soil Sci. 23: 271–302.

(11) ———, CARTER, E. G., and GOLDTHORPE, H. C.
1919. INFLUENCE OF SALTS ON THE NITRIC-NITROGEN ACCUMULATION IN THE SOIL. Jour. Agr. Research 16: 107–135, illus.

(12) KELLEY, W. P.
1916. NITRIFICATION IN SEMIARID SOILS—I. Jour. Agr. Research 7: 417–437.

(13) LIPMAN, CHAS. B.
1912. TOXIC EFFECTS OF "ALKALI SALTS" IN SOILS ON SOIL BACTERIA. II. NITRIFICATION. Centbl. Bakt. [etc.] (II) 33: 305–313, illus.

(14) LIPMAN, C. B., and BURGESS, P. S.
1914. ANTAGONISM BETWEEN ANIONS AS AFFECTING SOIL BACTERIA. II. NITRIFICATION. Centbl. Bakt. [etc.] (II) 41: 430–444, illus.

(15) NEHRING, K., and MÖBIUS, H.
1934. ÜBER DEN EINFLUSS DER ZUSAMMENSETZUNG DES SORPTION-KOMPLEXES DER BÖDEN AUF FESTLEGUNG DER VERSCHIEDENEN AMMONIUMVERBINDUNGEN. Ztschr. Pflanzenernähr., Düngung u. Bodenk. 38: 294–333.
(16) WAKSMAN, SELMAN A.
1932. PRINCIPLES OF SOIL MICROBIOLOGY. Ed. 2, thoroughly rev., 894 pp., illus. Baltimore.
(17) ———
1936. HUMUS; ORIGIN, CHEMICAL COMPOSITION, AND IMPORTANCE IN NATURE. 494 pp., illus. Baltimore.

AN APPARENT INDUCED LOSS OF NITROGEN-FIXING ABILITY IN AZOTOBACTER[1]

By CHARLES R. STUMBO, formerly *graduate assistant in bacteriology*, and P. L. GAINEY, *soil bacteriologist, Kansas Agricultural Experiment Station*

INTRODUCTION

There is abundant evidence that *Azotobacter* will not make use of free atmospheric nitrogen in its metabolism if it has at its disposal an adequate quantity of available combined nitrogen. Such being the case, it does not seem unreasonable to suppose that if cultures of the organism were grown under conditions where the utilization of atmospheric nitrogen was completely suppressed for a sufficient period the organism might lose its ability to metabolize free nitrogen. Such an alteration in the metabolism of *Azotobacter* would be of interest from a practical as well as from a theoretical point of view since in fertile soils a concentration of available nitrogen sufficient to inhibit fixation is frequently present for considerable periods of time. Briscoe [2] has recorded a limited amount of data indicating that some such change does take place; she found that after prolonged growth on media high in nitrate nitrogen some strains of *Azotobacter* did not grow normally when transferred to a medium low in fixed nitrogen. The primary objectives of the present investigation were to verify Briscoe's work and to ascertain whether the failure of *Azotobacter* to grow normally on a nitrogen-low medium after culture at high concentrations of nitrate nitrogen could be attributed to a loss of its ability to metabolize free nitrogen.

PROCEDURE

Pure cultures of *Azotobacter chroöcoccum* (all strains studied produced a black pigment and were arbitrarily classed as *chroöcoccum*) were isolated from local soils and grown both on nitrogen-free [3] mannite agar and on the same medium containing various concentrations of nitrate nitrogen as potassium nitrate. Transfers were made every 3 or 4 days and incubation was at 28° to 30° C. In this way it was possible to determine the ability of any particular strain to tolerate nitrate nitrogen. After various intervals, transfers were made from the highest concentrations of nitrate nitrogen agar upon which normal (macroscopically equal to growth on nitrogen-free agar) growth took place to nitrogen-free agar, and to agar containing very low concentrations of nitrate nitrogen. By comparing the growth of the various strains on (1) the high nitrate nitrogen agar, (2) the high nitrate nitrogen agar cultures transferred to nitrogen-free agar, and (3) the

[1] Received for publication December 31, 1937; issued August 1938.

[2] BRISCOE, FAITH WINIFRED. THE EFFECT OF AVAILABLE NITROGEN UPON THE GROWTH AND NITROGEN-FIXING ABILITY OF AZOTOBACTER. Master Sci. Thesis, Kansas State College Library. 1933.

Contribution No. 171, Department of Bacteriology, Kansas Agricultural Experiment Station.

[3] "Nitrogen-free" as used in this paper refers to agar that contained no more than 5 to 10 p. p. m. of combined nitrogen.

same strain grown continuously on nitrogen-free agar, it was possible to note any marked change in the growth of a given strain on nitrogen-free agar resulting from previous prolonged growth under conditions where nitrogen fixation was suppressed. More than 150 strains were studied in this way.

PRELIMINARY DATA

In a recent paper from this laboratory [4] data were submitted relative to the tolerance of *Azotobacter chroöcoccum* to nitrate nitrogen. These data indicated considerable strain variation in this respect, but most of the strains tested grew readily on mannite agar containing 3,000 to 4,000 parts per million of nitrate nitrogen. Additional strains have been tested and the results previously reported are amply verified by the data obtained.

Attention was also called in the above-mentioned paper to an apparently greater toxicity of the nitrate ion when cultures were transferred at infrequent intervals, a number of cultures having been accidentally lost by failure to transfer at the regular 3- to 4-day interval. Additional information was sought on this point and sufficient data relative thereto are on record to prove that the earlier observations were correct.

In table 1 are presented data showing the concentrations of nitrate nitrogen tolerated by one series of 46 freshly isolated and 3 previously isolated strains, together with records of growth of each strain on nitrogen-free and on 100 p. p. m. nitrate nitrogen agar, after having been grown on the high nitrate nitrogen agar for 105 days. The 1,000, 3,000, and 4,000 p. p. m. of nitrate nitrogen were the highest concentrations tested upon which the various strains listed thereunder would consistently produce normal growth. The term "normal" as here used merely means that the growth appeared macroscopically to equal that of the same culture growing continuously on the nitrogen-free agar. The concentration of 100 p. p. m. of nitrate nitrogen agar was employed because previously reported investigations (Burk et al.) [5] indicated that this was approximately the minimum concentration that would supply adequate nitrogen for maximum growth without the assimilation of atmospheric nitrogen.

In subsequent discussions the cultures that were kept continuously on nitrogen-free agar will be referred to as the 0 cultures, while those maintained on 1,000, 3,000, 4,000, etc., p. p. m. of nitrate nitrogen agar will be referred to as the 1,000 p. p. m., 3,000 p. p. m., 4,000 p. p. m., etc., cultures.

From the data presented in table 1 it is evident that all 1,000, 3,000, and 4,000 p. p. m. cultures grew readily when transferred to 100 p. p. m. nitrate nitrogen agar, but that 18 of the 49 cultures tested in this instance failed to grow or grew very poorly on the nitrogen-free agar. The difference in the growth of strain 4,000 p. p. m. O–2 and 0 O–2 upon a nitrogen-free agar is illustrated in figure 1. The number of strains growing abnormally on nitrogen-free agar, following growth on high nitrate nitrogen agar, was considerably larger than noted in previous tests. In view of the fact that the so-called nitrogen-free agar actually contained from 5 to 10

[4] GAINEY, P. L. THE TOLERANCE OF NITRATE BY PURE CULTURES OF AZOTOBACTER. Soil Sci. 42: 445–459. 1936.

[5] BURK, DEAN, LINEWEAVER, HANS, and HORNER, C. KENNETH. THE SPECIFIC INFLUENCE OF ACIDITY ON THE MECHANISM OF NITROGEN FIXATION BY AZOTOBACTER. Jour. Bact. 27: 325–340, illus. 1934.

p. p. m. of nondializable nitrogen, it was thought that the limited growth observed might have resulted from the utilization of this nitrogen.

TABLE 1.—*Relative growth of 49 cultures of Azotobacter in media containing 0 and 100 p. p. m. of nitrate nitrogen following 105 days' growth at higher concentrations of nitrate nitrogen*

1,000 p. p. m.[1]			3,000 p. p. m.[1]			4,000 p. p. m.[1]		
Culture	Growth on nitrogen-free agar [2]	Growth at 100 p. p. m. nitrate nitrogen	Culture	Growth on nitrogen-free agar	Growth at 100 p. p. m. nitrate nitrogen	Culture	Growth on nitrogen-free agar	Growth at 100 p. p. m. nitrate nitrogen
K-1	4	4	E-3	3	3	D-3	3	3
K-2	4	4	C-4	4	4	I-1	3	3
H-1	4	4	J-6	4	4	I-2	?	3
F-1	4	4	O-3	3	3	I-3	3	3
J-2	?	3	1	?	3	I-4	4	4
B-1	?	3	4	?	2	I-5	3	3
B-3	?	4	6a [3]	?	3	I-6	3	3
B-4	4	4	E-1	3	3	J-5	4	4
H-3	4	4	G-2	4	4	N-2	3	3
A-4	?	4	G-3	4	4	N-1	4	4
F-2	?	3	G-1	4	4	N-4	?	2
C-1	4	4	H-2	3	3	O-2	?	3
C-2	?	3	J-1	?	3	P-1	?	2
C-3	4	4	J-3	3	3	P-3	?	3
N-3	1	1	L-1	3	3	5b [3]	?	3
0	4	4	N-5	4	4			
L-2	?	4						
5a [3]	?	4						

[1] Concentration of nitrate nitrogen at which *Azotobacter* was cultured for 105 days.
[2] ? indicates slight or no visible growth after 3 days; 1, 2, 3, and 4 indicate increasing quantities of growth, 4 representing normal growth of the strain in question when cultured on nitrogen-free agar continuously.
[3] Cultures grown at higher concentration for approximately 7 months.

It is obvious from these results that some change had taken place in the physiological make-up of certain strains of *Azotobacter* as a result of their being cultured on an agar medium of high nitrate nitrogen content. Subsequent tests proved that if the quantity of nitrate nitrogen furnished the 18 "abnormal" strains was inadequate to supply the needs of the organism, growth of young cultures was more or less proportional to the available nitrogen, whereas, growth of the other 31 strains was independent of the fixed nitrogen supplied. The most obvious inference to be drawn from these results is that the abnormal strains had partly or completely lost their ability to utilize atmospheric nitrogen. Definite proof of such an alteration, is, however, not so easily obtained.

GROWTH AND NITROGEN FIXATION ON AGAR

Quantitative methods were resorted to in an effort to ascertain whether the very limited growth produced by the 18 abnormal cultures on nitrogen-free agar was due to the assimilation of the 5 to 10 p. p. m. of fixed nitrogen present in the medium and whether the treatment to which the cultures had been subjected had altered their ability to utilize free nitrogen.

Fifty cubic centimeters of nitrogen-free or low nitrate nitrogen mannite agar were placed in large sterile Petri dishes (5½ inches in diameter). These were inoculated in quadruplicate with 0.3 cc of a saline suspension (roughly standardized to McFarland's nephelometer tube No. 2) of 48-hour agar slant cultures. The contents of two of the dishes were immediately analyzed for total nitrogen while the other two were incubated at 28° to 30° C. for 3 to 4 days, after which their total nitrogen content was determined. The data secured from three such

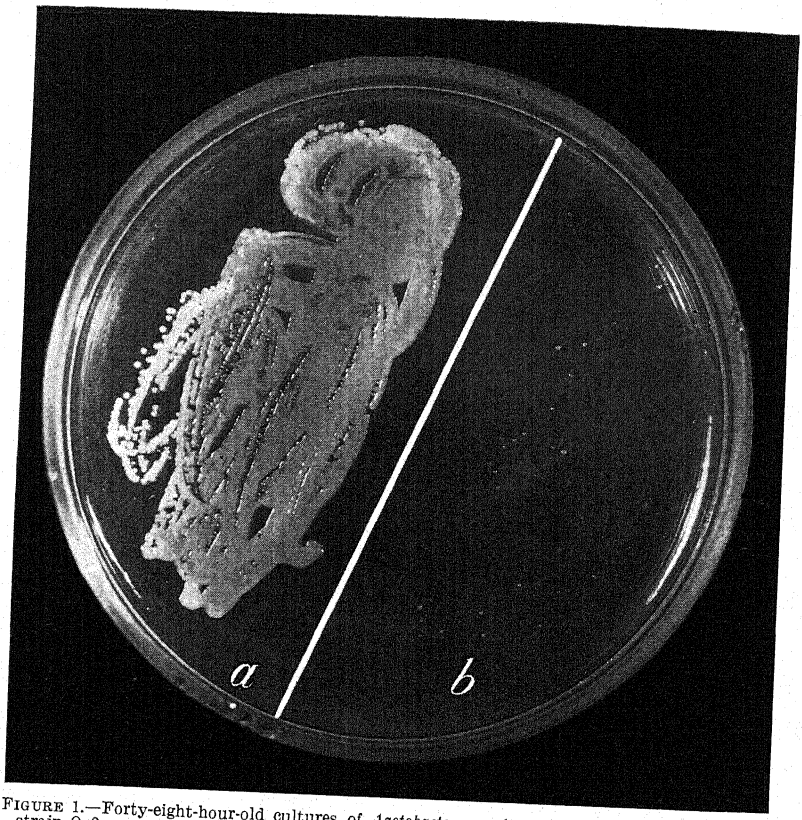


FIGURE 1.—Forty-eight-hour-old cultures of *Azotobacter* on nitrogen free agar: *a*, Culture of strain O–2 grown continuously on nitrogen free agar following isolation; *b*, the same strain as in *a* with several months preliminary culturing upon 4,000 p. p. m. nitrate-nitrogen agar.

tests with culture 4,000 p. p. m. 5b, three with culture 4,000 I–2, and one with culture 4,000 O–2 are recorded in tables 2 and 3. In parallel columns are indicated the mean relative growth, mean total nitrogen utilized, and mean atmospheric nitrogen fixed. The quantity of free atmospheric nitrogen metabolized is the factor of major importance, and the significance of the values recorded have been determined statistically according to Fisher's [6] method where limited replications are available. The significance of the mean values for nitrogen fixed is recorded in terms of P, where P represents the probability of a value equal to that recorded being due to chance. For example, if for the recorded mean difference in nitrogen content between the

[6] FISHER, RONALD AYLMER, STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 5, rev. and enl., 319 pp., illus. Edinburgh and London. 1934.

controls and treated samples $P=0.4$, then the probability is that 4 times out of 10 a value equal to that recorded will be due to chance alone. Differences for which the value P is greater than 0.05 are not regarded as significant.

TABLE 2.—*Growth and nitrogen fixed by cultures 5b and I–2, on agar varying in nitrogen content, with an incubation period of 3 days*

CULTURE 5b

Experiment No. and calculated nitrate nitrogen added to media (p. p. m.)	Controls		4,000 p. p. m. culture [1]				0 culture [2]			
	Nitrate nitrogen per 50 cc media as determined	Total nitrogen per 50 cc media as determined	Observed growth	Total organic nitrogen per 50 cc media after growth	Nitrogen fixed per 50 cc media	P	Observed growth	Total organic nitrogen per 50 cc media after growth	Nitrogen fixed per 50 cc of media	P
Experiment 1:	*Mg*	*Mg*		*Mg*	*Mg*			*Mg*	*Mg*	
0	0	0.45	[3] ?	0.50	0.05	0.4	4	3.39	2.84	0.01
50	2.39	2.84	2	3.23	.39	.8	4	5.39	2.55	.01
100	4.80	5.25	3	6.20	.95	.01	4	6.50	1.25	.01
150	7.22	7.67	4	8.21	.54	.3	4	9.00	1.33	.01
Experiment 2:										
0	0	.48	1	1.26	.78	.01	4	4.41	3.93	.01
50	2.49	2.97	3	6.17	3.20	.01	4	6.34	3.39	.01
100	5.01	5.74	4	7.29	1.55	.01	4	7.71	1.97	.01
Experiment 3:										
0	0	.40	?	.56	.16	.2	4	5.61	5.21	.01
10	.51	.91	1	1.26	.35	.05				
25	1.36	1.76	2	2.51	1.15	.01				
50	2.62	3.02	3	4.07	1.05	.01				
100	5.46	5.86	4	6.75	.89	.01				
150	7.74	8.14	4	9.06	.92	.01				

CULTURE I–2

Experiment 1:										
0	0	0.45	1	1.29	0.84	0.01	4	6.35	5.90	0.01
50	2.39	2.84	4	4.31	1.47	.01	4	5.94	3.10	.01
100	4.80	5.25	4	6.66	1.41	.01	4	7.08	1.83	.01
150	7.22	7.67	4	8.03	.36	.05	4	9.00	1.33	.01
Experiment 2:										
0	0	.48	1	2.07	1.59	.01	4	8.34	7.86	.01
50	2.49	2.97	3	5.33	2.36	.01	4	7.01	4.04	.01
100	5.01	5.73	4	6.93	1.20	.01	4	7.50	1.77	.01
Experiment 3:										
0	0	.40	1	.84	.44	.01	4	10.02	9.62	.01
10	.51	.91	2	1.47	.56	.01				
25	1.36	1.76	3	3.12	1.36	.01				
50	2.62	3.02	4	5.55	2.53	.01				
100	5.46	5.86	4	8.62	2.76	.01				
150	7.74	8.14	4	10.31	2.17	.01				

[1] Previously grown at 4,000 p. p. m. nitrate nitrogen. See text for meaning of P.
[2] Previously grown on "nitrogen-free" agar.
[3] See footnote 2, table 1.

The quantity of nitrogen fixed was obtained by subtracting the total quantity of nitrogen originally present in the medium from that found at the end of the incubation period. This procedure seemed justifiable since in every instance except one qualitative tests for nitrate nitrogen, as well as the total quantity of organic nitrogen present, indicated that all nitrate nitrogen had been used up by the organisms.

The data presented in tables 2 and 3 indicate quite definitely certain facts, among which the following are of significance in this connection:

(1) The 0 culture of all three strains is capable of readily utilizing either free atmospheric or nitrate nitrogen.

TABLE 3.—*Growth and nitrogen fixed by culture O–2 on agar varying in nitrogen content, with incubation periods of 5 and 8 days*

Calculated nitrate nitrogen added to media (p. p. m.)	Controls		4,000 p. p. m. culture [1]							
	Nitrate nitrogen per 50 cc media as determined	Total nitrogen per 50 cc media as determined	Observed growth after—		Total organic nitrogen per 50 cc media after growth for—		Nitrogen fixed per 50 cc of media			
			5 days	8 days	5 days	8 days	5 days	*P*	8 days	*P*
	Mg	*Mg*			*Mg*	*Mg*	*Mg*		*Mg*	
0	0	0.24	[2] 1	1	0.28	0.28	0.04	0.2	0.04	0.2
2½	.12	.36	[2] 1	2	.48	.63	.12	.02	.27	.01
7½	.37	.61	[2] 1	2	.49	1.11	−.12	--------	.50	.01

Calculated nitrate nitrogen added to media (p. p. m.)	Controls		0 culture [3]							
	Nitrate nitrogen per 50 cc media as determined	Total nitrogen per 50 cc media as determined	Observed growth after—		Total organic nitrogen per 50 cc media after growth for—		Nitrogen fixed per 50 cc of media			
			5 days	8 days	5 days	8 days	5 days	*P*	8 days	*P*
	Mg	*Mg*			*Mg*	*Mg*	*Mg*		*Mg*	
0	0	0.24	4	4	5.54	5.76	5.45	0.01	5.67	0.01
2½	.12	.36	--------	--------	--------	--------	--------	--------	--------	--------
7½	.37	.61	--------	--------	--------	--------	--------	--------	--------	--------

[1] Previously grown at 4,000 p. p. m. of nitrate nitrogen. See text for meaning of *P*.
[2] See footnote 2, table 1.
[3] Previously grown on "nitrogen-free" agar.

(2) In the presence of less nitrate nitrogen than the culture is capable of making use of, the quantity of atmospheric nitrogen fixed decreases as the quantity of nitrate nitrogen increases. A concentration of 150 p. p. m. of nitrate nitrogen was inadequate to completely inhibit fixation in the few instances tested.

(3) Fixation of nitrogen by the 4,000 p. p. m. cultures on nitrogen-free agar occurred in the case of I–2 (three trials), did not take place in the case of O–2 (one trial), and was significant in only one out of three trials in the case of 5b.

(4) Fixation of nitrogen by the 4,000 p. p. m. cultures was stimulated in the case of all three strains by the addition of small quantities of nitrate nitrogen.

(5) Where fixation of atmospheric nitrogen was accomplished by a 4,000 p. p. m. culture on nitrogen-free agar, the quantities fixed were nearly always very small as compared with the quantities fixed by the O culture under identical conditions.

From these results it appears that the previous treatment of the 4,000 p. p. m. cultures of the three strains studied quantitatively had effected a radical alteration in the ability of the organism to metabolize free atmospheric nitrogen.

GROWTH AND NITROGEN FIXATION IN LIQUID MEDIA

Since the presence in the agar medium of limited quantities of nitrate nitrogen, even as small as 2½ p. p. m., stimulated fixation of

nitrogen by the 4,000 p. p. m. cultures, and the so-called nitrogen-free agar contained from 5 to 10 p. p. m. of combined nitrogen, it was thought that this nondializable nitrogen, present in the agar-agar, possibly might have acted as a stimulant to fixation in those instances in which fixation was noted on the nitrogen-free medium. It was, therefore, deemed advisable to undertake similar studies with a liquid medium in which the concentration of fixed nitrogen could be reduced much lower, i. e., 0.9 to 1.5 p. p. m. The same medium without the addition of the agar was employed.

Growth of *Azotobacter* in a stationary liquid medium takes place very slowly unless the layer of the liquid is extremely thin. In order to make use of sufficient media for quantitative purposes without employing relatively large cultural vessels it was necessary to resort to aeration of cultures as suggested by Hunter [7] for increasing the rate of growth.

One hundred cubic centimeters of the medium were placed in 300-cc pyrex Erlenmeyer flasks, fitted with an aeration tube inserted in a cork stopper well wrapped with cotton, and the flask with its contents was sterilized. The aeration tube was prepared by sealing the end of a piece of ordinary glass tubing and perforating it laterally a number of times near the sealed end, to facilitate distribution of the air.

Air under pressure was passed successively through similar aeration tubes inserted respectively in a bottle of weak sulphuric acid, a bottle of weak sodium hydroxide, a bottle of weak mercuric chloride, and two bottles of distilled water. The chief purpose in using the water was to saturate the air with moisture in order to reduce to a minimum the loss of the culture medium through evaporation.

The flasks were inoculated in replications of two to five (usually five) with one drop of a suspension of the organisms prepared as previously described. The quantity of nitrogen contained in one drop of the inoculum was so small that it could be ignored, hence the aerated controls were not inoculated.

It was found necessary to aerate rather vigorously, otherwise flocculation of the suspended bacteria appeared to take place, resulting in almost complete cessation of growth after 24 to 48 hours.

Two strains were studied sufficiently to give a general idea of the results that may be expected by such methods. The actual quantities of nitrogen fixed by different strains, however, were found in preliminary tests to vary all the way from those here recorded to normal. This was as expected, since any such alteration in nitrogen-fixing ability as here indicated might be expected to vary quantitatively both with the strain and with the period of preliminary culturing upon a high nitrate medium.

From the data presented in tables 4 and 5 it is evident that the 0 culture of both strains 5b and O–2 were active in metabolizing free nitrogen when grown in a suitable liquid medium, the latter fixing somewhat larger quantities than the former.

The 4,000 p. p. m. cultures were incapable of metabolizing more than traces of nitrogen during an incubation period of 4 to 5 days when grown in a nitrogen-free liquid medium—the largest quantity recorded for either culture being 0.12 mg as compared with from 2.91

[7] HUNTER, O. W. STIMULATING THE GROWTH OF AZOTOBACTER BY AERATION. Jour. Agr. Research 23: 665-677, illus. 1923.

to 7.05 mg for the 0 cultures under identical conditions. In only one instance in the 8 experiments of 4 to 5 days' duration could the fixation be regarded as definitely significant, and in this case the actual quantity fixed was only 0.12 mg by the 4,000 p. p. m. culture of strain 5b.

TABLE 4.—*Growth and nitrogen fixation by culture 5b in liquid media varying in nitrogen content*

Experiment No., incubation period and calculated nitrate nitrogen added to media (p. p. m.)	Controls		4,000 p. p. m. culture [1]				0 culture [2]			
	Nitrate nitrogen per 100 cc media as determined	Total nitrogen per 100 cc media as determined	Observed growth	Total organic nitrogen per 100 cc media after growth	Nitrogen fixed per 100 cc of media	*P*	Observed growth	Total organic nitrogen per 100 cc media after growth	Nitrogen fixed per 100 cc of media	*P*
Experiment 1; incubation period 4 days:	*Mg*	*Mg*		*Mg*	*Mg*			*Mg*	*Mg*	
0	0	0.15	[3] 0	0.21	0.06	0.3	4	4.31	4.16	0.01
0	0	.15	0	.18	.03	.6				
5	.49	.64	2	.89	.25	.05				
10	1.01	1.16	4	2.03	.87	.01				
20	2.17	2.32	4	3.50	1.18	.01				
Experiment 2; incubation period 4 days:										
0	0	.15	0	.20	.05	.1	4	3.06	2.91	.01
10	1.11	1.26	3	3.03	1.77	.01				
15	1.41	1.56	4	3.67	2.11	.01				
20	2.03	2.18	4	3.70	1.52	.01				
25	2.57	2.72	4	4.21	1.49	.01				
50	5.13	5.28	4	5.94	.66	.01				
75	7.54	7.69	4	7.92	.23	.3				
100	9.97	10.12	4	10.34	.22	.4				
125	12.77	12.92	4	12.77	−.15					
Experiment 3; incubation period 4 days:										
0	0	.09	0	.13	.04	.05				
2½	.25	.34	2	.52	.18	.01				
5	.50	.59	3	1.40	.81	.01				
7½	.75	.84	3	1.40	.56	.01				
10	1.00	1.09	3	1.40	.31	.02				
12½	1.25	1.34	3	1.52	.18	.04				
15	1.50	1.59	3	1.83	.24	.03				
17½	1.75	1.84	3	2.10	.26	.01				
Experiment 4; incubation period 5 days:										
0	0	.09	0	.15	.06	.2	4	5.38	5.29	.01
2½	.25	.34	4	1.86	1.62	.01				
5	.50	.59	4	2.23	1.64	.01				
7½	.75	.84	4	2.63	1.79	.01				
10	1.00	1.09	4	2.93	1.84	.01				
12½	1.25	1.34	4	3.07	1.73	.01				
15	1.50	1.59	4	2.23	.64	.01				
Experiment 5; incubation period 5 days:										
0	0	.09	?	.21	.12	.01	4	4.85	4.76	.01
2½	.25	.34	3	1.28	.94	.01				
5	.50	.59	4	2.11	1.52	.01				
7½	.75	.84	4	2.40	1.56	.01				
10	1.00	1.09	4	1.72	.63	.01				
12½	1.25	1.34	4	2.10	.66	.01				
15	1.50	1.59	4	2.29	.45	.01				
Experiment 6; incubation period 8 days:										
0	0	.09	1	1.09	1.00	.01	4	5.80	5.71	.01
2½	.25	.34	4	3.20	2.86	.01				
5	.30	.59	4	3.00	2.41	.01				
7½	.75	.84	4	4.06	3.22	.01				
10	1.00	1.09	4	3.44	2.35	.01				
12½	1.25	1.34	4	4.27	2.93	.01				
15	1.50	1.59	4	4.67	2.83	.01				

[1] Previously grown at 4,000 p. p. m. of nitrate nitrogen. See text for meaning of *P*.
[2] Previously grown on "nitrogen-free" agar.
[3] See footnote 2, table 1.

If the incubation period was lengthened to 8 days, culture 4,000 p. p. m. 5b fixed definite quantities of nitrogen in a nitrogen-free liquid medium, though still much smaller than the 0 5b culture fixed in 4 days. Culture 4,000 p. p. m. O–2 failed to fix significant quantities of nitrogen in a nitrogen-free medium even during an 8-day incubation period.

TABLE 5.—*Growth and nitrogen fixation by culture O–2 in liquid media varying in nitrogen content, with incubation periods of 4 and 8 days*

Experiment No. and calculated nitrate nitrogen added (p. p. m.)	Controls		4,000 p. p. m. culture [1]							
	Nitrate nitrogen per 100 cc media as determined	Total nitrogen per 100 cc media as determined	Observed growth after—		Total organic nitrogen per 100 cc of media after growth for—		Nitrogen fixed per 100 cc of media			
			4 or 5 days	8 days	4 or 5 days	8 days	4 or 5 days	*P*	8 days	*P*
Experiment 1:	*Mg*	*Mg*			*Mg*	*Mg*	*Mg*		*Mg*	
0	0	0.09	[3] ?	?	0.12	0.09	0.03	0.2	0	
2½	.25	.34	1	1	.32	.47	−.02		.13	0.02
7½	.75	.84	2	2	1.01	.89	.17	.02	.05	.3
Experiment 2:										
0	0	.09	?	?	.12	.10	.03	.3	.01	.8
2½	.25	.34	1	1	.33	.25	−.01		−.09	
7½	.75	.84	2	2	.94	.87	.10	.05	.03	.2
Experiment 3:										
0	0	.09	?	?	.09	.09	0		0	
2½	.25	.34	?	?	.09	.09	−.25		−.25	
7½	.75	.84	1	1	.09	.36	−.75		−.48	

Experiment No. and calculated nitrate nitrogen added (p. p. m.)	Controls		4,000 p. p. m. culture [2]							
	Nitrate nitrogen per 100 cc media as determined	Total nitrogen per 100 cc media as determined	Observed growth after—		Total organic nitrogen per 100 cc of media after growth for—		Nitrogen fixed per 100 cc of media			
			4 or 5 days	8 days	4 or 5 days	8 days	4 or 5 days	*P*	8 days	*P*
Experiment 1:	*Mg*	*Mg*			*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*
0	0	0.09	4	4	7.14	7.26	7.05	0.01	7.17	0.01
2½	.25	.34								
7½	.75	.84								
Experiment 2:										
0	.00	.09	4	4	6.45	6.60	6.36	.01	6.51	.01
2½	.25	.34								
7½	.75	.84								
Experiment 3:										
0	0	.09	4	4	5.73	6.37	5.64	.01	6.28	.01
2½	.25	.34								
7½	.75	.84								

[1] Previously grown as 4,000 p. p. m. nitrate nitrogen. See text for meaning of *P*.
[2] Previously grown on "nitrogen-free" agar.
[3] See footnote 2, table 1.

When the liquid medium contained fixed nitrogen in the form of potassium nitrate, the 4,000 p. p. m. cultures of both 5b and O–2 grew readily and transformed the nitrate nitrogen into organic nitrogen. The quantity of free nitrogen metabolized by the 0 5b culture was small compared with the quantity of combined nitrogen metabolized by the 4,000 p. p. m. 5b culture.

The presence of very small (2½ p. p. m.) quantities of nitrate nitrogen in the medium not only made it possible for the 4,000 p. p. m. culture of strain 5b to grow but also enabled it to readily metabolize free atmospheric nitrogen. Increasing the quantity of combined nitrogen, within a limited range, i. e., up to 5 to 20 p. p. m., increased the ability of this culture to utilize atmospheric nitrogen in every experiment. Further increases, however, resulted in decreased fixation until fixation reached zero, when an adequate quantity was present to supply the needs of the organisms.

In no instance, even when incubation was extended to 8 days, was the 4,000 p. p. m. culture of strain O–2 observed to fix significant quantities of nitrogen when grown in a liquid nitrogen-free medium. Even when small quantities of nitrate nitrogen were added to the medium, fixation was so small and irregular as to suggest that some other factor might have been responsible for the apparent significant increases in nitrogen here recorded.

The almost complete absence of nitrogen fixation in the 4,000 p. p. m. O–2 cultures raised the question of the possibility of the *Azotobacter* having been supplanted by a contaminant so similar morphologically and culturally as to have escaped notice. However, reisolations from liquid cultures upon agar, even though initial growth was very slow, followed by numerous transfers, gradually brought the culture back to where definite fixation of nitrogen could be demonstrated, proving conclusively that the culture was a modified strain of *Azotobacter*.

DISCUSSION AND SUMMARY

It is evident from the data presented that prolonged growth of *Azotobacter* in the presence of relatively high concentrations of potassium nitrate resulted in such an alteration in the physiological make-up of certain strains of the organism as to render them incapable of growing normally in the absence of combined nitrogen. Such a condition was reflected (1) in very limited or no visible growth, (2) inability to metabolize atmospheric nitrogen, or (3) a combination of both conditions. It is believed that the effect of the potassium nitrate was due to the nitrogen since potassium ions supplied in other salts did not have a similar effect. The discussion is restricted for the most part to nitrate nitrogen because the experiments were largely concerned with this form. Limited experimentation with other nitrogenous compounds indicated that they would give similar results provided the nitrogen was available to *Azotobacter*.

All strains that did not grow on the nitrogen-free medium grew when small quantities of potassium nitrate were added to the medium. In the case of certain strains such growth, following previous growth at high concentrations of nitrate nitrogen, was proportional to the added nitrogen, and quantitative data proved the absence of nitrogen fixation. In the case of other strains the presence of very small quantities of nitrate nitrogen sufficed to initiate growth and subsequent growth was more or less independent of the quantity of nitrogen initially present. Quantitative data proved in such instances that atmospheric nitrogen was readily utilized. In the former case the organisms apparently lost, at least temporarily, their ability to fix free nitrogen, whereas in the latter case this ability was conditioned

upon growth and was readily regained when favorable growth conditions were reestablished. In other words, very limited quantities of combined nitrogen served to restore some, but not all, strains to normal within a very short time.

Just what alteration occurred in the physiological make-up of those strains that underwent the suggested change is not known. The available evidence indicates temporary or more or less permanent loss of the ability to metabolize atmospheric nitrogen. As a possible explanation of the change it might be assumed that when grown under conditions where nitrogen fixation is completely suppressed for an adequate length of time, the organisms cease to elaborate the nitrogen-fixing enzyme system. If the further assumption is made that combined nitrogen is essential for the elaboration of the enzymes necessary in nitrogen fixation it can be easily understood why the presence of limited quantities of nitrate nitrogen restored the free-nitrogen metabolizing ability in those strains regaining normalcy in its presence. It is also not inconceivable that even the ability to elaborate the enzyme system was lost by those strains that failed to return to normal in the presence of low concentrations of nitrate nitrogen.

THE REACTIONS OF WIREWORMS TO ARSENICALS[1]

By CHARLES E. WOODWORTH[2]

Associate entomologist, Division of Truck Crop and Garden Insect Investigations, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture

INTRODUCTION

It has been noted (*4*, *7*)[3] that arsenicals in bran or whole-wheat baits render these baits relatively unattractive to wireworms. It has also been noted that the mortality of wireworms captured by this method is very little more than when unpoisoned baits are used. Since arsenic is generally very toxic to insects, these observations have aroused considerable speculation. The present investigation was designed to explain the failure of baits poisoned with arsenic to attract and kill wireworms, and to obtain a better understanding of the mode of entry of arsenic into the body of the larva. The Pacific coast wireworm (*Limonius canus* Lec.) was selected for this study, as it is one of the four most important species, from an economic standpoint, in the irrigated lands of the Pacific Northwest. The other three species, *L. californicus* (Mann.), *L. subauratus* Lec., and *L. infuscatus* Mots., belonging as they do to the same genus, differ only slightly in morphology and physiology from the species selected; so the results are for the most part applicable to all four species.

EXPERIMENTAL PROCEDURE

TESTS WITH CORNSTARCH PELLETS CONTAINING ARSENICALS

In order to study the effects of arsenic upon wireworms, larvae were confined in glass cages with cornstarch pellets containing an arsenical. The pellets were made of 4 parts of cornstarch and 1 part of the arsenical, moistened with sufficient distilled water to prevent crumbling. Cornstarch was used because it is an attractive food for this species and does not get hard after it is wet, as does wheat starch. The arsenicals used were acid lead arsenate, paris green, mercurous arsenite, acid sodium arsenate, and sodium arsenite. Unpoisoned cornstarch pellets were used as checks. Cornstarch made alkaline to pH 10 with sodium hydroxide was also used as a check to determine whether the alkalinity of sodium arsenate and sodium arsenite had any effect on their repellency. The other arsenicals added to the cornstarch were either acid or neutral. Fast green to a concentration of about 0.01 percent was sometimes added to the mixtures, as this material could be seen through the body wall and was found to be nontoxic. The mixtures were cast in $\frac{3}{16}$-inch holes in a piece of sheet iron three thirty-seconds of an inch thick. The pellets were pushed out with a sawed-off nail and allowed to dry in air. For these studies 256 larvae were used, including the checks, and were kept

[1] Received for publication Feb. 7, 1938; issued August 1938.

[2] The author is indebted to M. C. Lane, in charge of wireworm investigations in the Pacific Northwest, Walla Walla, Wash, under whose direction the work was conducted.

[3] Italic numbers in parentheses refer to Literature Cited, p. 237.

individually in the cages with the pellets. All the dead wireworms and an adequate sample of the live ones from each group were tested for arsenic.

TESTS WITH CORNSTARCH PELLETS CONTAINING NONARSENICALS

Several nonarsenicals, both liquids and solids, were also incorporated in cornstarch pellets and placed in glass cages with the wireworms to determine whether the materials were ingested, and, if so, in what form. Finely ground graphite or lampblack, both inert substances, was mixed in the ratio of 1 part to 10 parts of cornstarch. The former was of a particle size that was easily recognizable under the microscope, and the latter colored the gastric content when taken in sufficient quantity. Iodine at a concentration of 0.1 percent and neutral red and Sudan III at a concentration of 0.05 percent were also used. Iodine stains starch a deep blue black and gives the grains their characteristic markings. Neutral red is a nontoxic dye. Sudan III is a fat-specific dye which gives a pink color to fats. Any of these stains should therefore be easily recognizable in the gastric content. Examination for these materials was made under a binocular microscope during the dissection of the wireworms and under the compound microscope in the study of the digestive-tract content. Starch was searched for by the familiar iodine test (*2, p. 93*) and reducing sugar by the Benedict test (*2, p. 744*). A total of 91 wireworms was used in these tests.

The cages that were used for the tests consisted of two microscope slides held apart by channeled metal supports and filled with a pad of moist cellucotton.[4] A cornstarch pellet was placed in one corner of each cage. The humidity of the atmosphere in the cages was maintained at about 100 percent by moistening the cellucotton pad and keeping the cages in a desiccator jar containing a little distilled water. The temperature was kept constant at 68° F. The period of confinement varied, but was usually more than 96 hours and occasionally as long as 1 month.

TESTS FOR ARSENIC

Tests for arsenic were made in a modified Gutzeit apparatus (*1, p. 371*), in which the reaction chamber was a 12- by 35-mm shell vial and the rest of the apparatus was reduced proportionately. The scrubber was a piece of 12-mm glass tubing 5 cm long and narrowed to 6 mm at the lower end, filled with loosely packed absorbent cotton that had been soaked in saturated lead acetate and squeezed free of excess liquid. The test papers were cut 2 by 100 mm, soaked in 4-percent alcoholic mercuric bromide solution for 30 minutes, and dried in air for about 20 minutes immediately before being used. The prepared paper strip was held in an 8-cm length of 4-mm glass tubing. All connections were made with the lower half of No. 00 rubber stoppers. The sensitivity was found to be about 1 microgram of arsenic. Blanks and checks of either 0.05 or 0.10 cc of 0.001 *N* sodium arsenite were run with each test. The checks were compared with a set of standards before the results were recorded.

Because of the proteinaceous and fatty nature of the insect material, an alkaline digestion was used rather than the acid one usually recommended. The procedure was as follows: The dissected material and

[4] WOODWORTH, C. E. A METAL AND GLASS INSECT CAGE. U. S. Bur. Ent. and Plant Quarantine, Multigraphed Cir. ET-69, 3 pp., illus. 1935.

five drops of 15-percent sodium hydroxide were placed in the shell-vial reaction chambers and held in a water bath for 30 minutes at a temperature just below the boiling point. Five drops of concentrated hydrochloric acid were then added. The mixture was allowed to stand 10 minutes at room temperature. Two drops each of 15-percent potassium iodide and 40-percent stannous chloride solution made up with concentrated hydrochloric acid were then added. The concoction was allowed to stand for another 10 minutes, after which about 0.33 g of zinc was added and the rest of the apparatus, which had been assembled, was immediately put in place. Precautions were taken to use arsenic-free reagents throughout the experiment.

TESTS WITH ARSENICAL SOLUTIONS

Experiments were also conducted in which the wireworms were submerged in solutions of sodium arsenite and acid lead arsenate and in distilled water. The concentrations of sodium arsenite that were used were 0.25, 0.5, 1, and 2 percent. For lead arsenate a saturated solution was used, which was found to contain about 0.75 microgram of arsenic per cubic centimeter. These solutions were put in 12- by 60-mm shell vials, 2.5 cc per vial. One wireworm was submerged in each vial, and groups of 10 were kept for different periods of time at a temperature of 68° F. They were then tested for gain in weight and survival or dissected and tested for arsenic. The lead arsenate was used only in preliminary survival tests.

In the experiment to determine the gain in weight during submergence 350 wireworms were used. These were divided into 7 groups of 10 each for each of the 4 concentrations of sodium arsenite and for the distilled water. All larvae were weighed before submergence, and those of group 1 at the end of 3, 6, 12, 24, 48, 96, and 192 hours. The other six groups were weighed only once or twice after submergence, to offset the effects of repeated handling. The two groups that were weighed twice were weighed at 6 and 192 hours and at 12 and 384 hours. All the wireworms in the last six groups were set aside after their final weighing to be tested for survival.

In the dissection tests wireworms were submerged in a 2-percent sodium arsenite solution for various periods of time. They were then washed, the blood taken, and dissected. The various tissues were tested for arsenic. The blood was obtained by piercing the integument, usually in the pleura of the prothorax, and then pressing the abdomen slightly until the blood came through the hole and stood out as a drop. The blood was readily removed by use of a capillary tube and transferred to the Gutzeit apparatus for testing. The question arose as to whether the blood might not get its arsenic from the integument. To answer this question a drop of distilled water was used to wash the larva just before the blood was taken, and this water was tested for arsenic. In all, 135 tests were made, though not all tissues were tested each time. Frequently just the blood and the digestive tracts were tested, and all the earlier tests were made without testing the wash water. Each test was made with only one wireworm.

RESULTS

TESTS WITH CORNSTARCH PELLETS CONTAINING ARSENICALS

The results of the tests with cornstarch pellets containing arsenic are shown in table 1. At first it appeared that it would be very easy

to obtain the median lethal dose, as the wireworms usually burrowed into the poisoned pellets and apparently fed. They quickly riddled the cellucotton pads with their burrowing and at times molted in the cages. In all but the sodium arsenite and sodium arsenate groups a large proportion of the pellets were riddled. The repellency was about 10 percent for the unpoisoned pellets, 24 percent for the lead arsenate groups, 31 percent for the mercurous arsenite groups, 37 percent for the paris green groups, 75 percent for the sodium arsenite groups, and 82 percent for the sodium arsenate groups. The alkalinity of the sodium salts of arsenic was not the cause of their high repellency, since the larvae that were placed with sodium hydroxide (pH 10) exhibited only a 30-percent repellency.

In all cases where fast green was used, the dye spread by capillary action from the pellet into the cellucotton, in which the wireworms moved. Tests for arsenic on portions of some of the pads from the sodium arsenite cages showed that the arsenical also had spread into the pads. In one case it had reached a concentration of 0.05 mg per square centimeter of pad at the far end of the cage.

In the sodium arsenate and sodium arsenite groups the attack was very slight; only 2 of the 19 wireworms that attacked the pellets were dead, and these contained arsenic in the digestive tract but not in the blood. In all but one case where arsenic was found in the blood the quantity was large. It was also found in these wireworms in readily measurable quantities in the muscle and fat, but was entirely lacking in the digestive tract. In the one case where arsenic was found in the blood of a living wireworm the quantity was minimal and barely recognizable. Similar sublethal quantities were found in the digestive tract of four live individuals.

TABLE 1.—*Results obtained in tests on wireworms confined with arsenical pellets*

Material added to cornstarch	Wireworms used	Pellets attacked	Wireworms showing fast green in digestive tract	Live wireworms not tested for arsenic [1]	Wireworms tested					
					No arsenic found		Arsenic found			
							In digestive tract		In blood	
					Alive	Dead	Alive	Dead	Alive	Dead
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
Lead arsenate	29	20	------	17	9	1	0	1	1	0
Lead arsenate and fast green	20	17	6	10	10	0	0	0	0	0
Paris green	25	15	------	13	12	0	0	0	0	0
Paris green and fast green	18	12	1	8	9	1	0	0	0	0
Mercurous arsenite	8	6	------	2	5	0	1	0	0	0
Mercurous arsenite and fast green	8	5	1	4	3	0	1	0	0	0
Sodium arsenate	25	4	------	10	7	0	0	1	0	7
Sodium arsenate and fast green	19	4	1	9	8	0	1	0	0	1
Sodium arsenite	25	6	------	7	13	0	0	1	0	4
Sodium arsenite and fast green	19	5	1	9	4	0	1	0	0	5
Checks:										
Cornstarch alone	25	23	------	13	10	2	0	0	0	0
Fast green	25	22	12	15	9	1	0	0	0	0
Sodium hydroxide (pH 10)	10	7	------	9	0	1	0	0	0	0
Total	256	146	22	126	99	6	4	3	1	17

[1] All dead wireworms were tested for arsenic.

TESTS WITH CORNSTARCH PELLETS CONTAINING NONARSENICALS

The results of the tests with nonarsenical pellets are shown in table 2. Of the 25 wireworms that received cornstarch alone, the 9 that are recorded as giving negative results either failed to feed or gave negative tests for starch when the content of the midintestine was studied. The iodine test did not reveal starch in the digestive tract even though the pellets showed clearly that a portion of the starch had been removed. The sugar test failed to demonstrate the presence of sugar in the digestive-tract content, though this might be accounted for by the small quantity of material available for the tests. The 16 doubtful cases were those individuals that were used as checks in other experiments and were not tested specifically for starch.

Of the 25 wireworms that were given access to the cornstarch and fast green, the 9 reported as doubtful were those that were not dissected and in which fast green was not visible through the integument, though the pellets had been attacked. Fast green is a hydrogen-ion indicator, the range being comparable with that of methyl red. There was no indication of an alkaline reaction in any part of the digestive tract. The doubtful cases with lampblack, neutral red, and Sudan III were those in which the color in the digestive tract was not sufficiently intense. Sudan III colored the fat bodies of all wireworms confined with this material, but was not definitely recognizable in the digestive tract in any case. The two doubtful cases with iodine were those in which a brown coloration was noted in the tract but it was not definitely caused by the iodine. This material appears to be repellent to wireworms.

TABLE 2.—*Results of tests on wireworms confined with nonarsenical pellets*

Material added to cornstarch	Wireworms used	Pellets attacked	Dead wireworms	Wireworms in which material was recognizable in digestive tract		
				Positive	Doubtful	Negative
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
None	25	23	2	0	16	9
Fast green	25	22	1	12	9	4
Graphite	10	6	0	0	0	10
Lampblack	12	9	1	5	3	4
Iodine	8	4	1	0	2	6
Neutral red	7	4	0	2	1	4
Sudan III	4	4	0	0	2	2

TESTS WITH ARSENICAL SOLUTIONS

It was noted in the experiments with the pellets that arsenic entered the blood but not the digestive tracts of wireworms that were on saturated cellucotton pads in which arsenic was found to be present. In preliminary trials submerged wireworms gained weight very rapidly, some larvae more than doubling their weight in 2 weeks. Table 3 gives the results of some experiments designed to determine the rate of increase in weight of larvae after various periods of submergence in sodium arsenite solution. The wireworms soon became bloated and turgid, and after 4 days certain individuals were hard to handle because of the danger of causing a rupture. As they were weighed in

groups of 10, a single ruptured individual would cause a considerable drop in the average weight. Dissection of some of the bloated wireworms showed that the digestive tract was slightly swollen, but not in proportion to the rest of the body. Mortality studies showed that the rate of killing was proportional to the time of exposure and the concentration of sodium arsenite. Saturated solutions of lead arsenate did not kill 100 percent of the wireworms even after submergence for a week. The mortality of the larvae in this material was comparable with that in distilled water for the same period.

TABLE 3.—*Rate of increase in weight of wireworms submerged in sodium arsenite solutions*

Concentration of sodium arsenite	Average original weight of wireworms	Average gain in weight of wireworms after submergence for—							
		3 hours	6 hours	12 hours	24 hours	48 hours	96 hours	192 hours	384 hours
Percent	*Milligrams*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
[1] 0.00	24.2	14.1	23.3	38.8	58.3	75.1	76.9	77.7	[2] 67.4
.25	24.1	15.7	24.0	35.0	62.6	71.2	74.9	[2] 72.5	[2] 84.7
.50	24.3	11.6	22.5	40.5	65.6	71.8	84.2	85.2	[2] 72.0
1.00	24.3	10.8	20.0	36.3	46.5	69.2	78.8	82.8	88.4
2.00	24.1	9.1	14.0	28.0	45.0	65.8	[2] 62.1	[2] 80.1	[2] 63.8

[1] Distilled water.
[2] 1 or more individuals ruptured.

TABLE 4.—*Quantity of arsenic found in parts of wireworms that had been submerged in 2-percent sodium arsenite solution*

Part tested	Arsenic found [1] after submergence for—						
	3 hours	18 hours	24 hours	48 hours	96 hours	144 hours	192 hours
Wash water	None	(2)	Trace	Trace	None	(2)	Trace.
Blood	Little	Much	Much	Much	Much [3]	Much [3]	Much.[3]
Fat	Trace	Little	Little	Little	do	Little	Do.
Digestive tract	do	None	None	None	Trace	Trace	Trace.
Nerve	do	do	do	Trace	None	None	None.
Heart	None	do	Trace	do	Trace	do	Do.
Tracheae	do	do	None	None	None	do	Do.
Muscle	Trace	Trace	Little	Little	Little	Little	Much.
Integument	do	(2)	do	do	do	do	Little.

[1] "Trace indicates 2 micrograms or less; "little," more than 2 micrograms and less than 10 micrograms; "much," 10 or more micrograms.
[2] Not tested.
[3] More than could be recorded on test paper.

Since the increase in weight of the submerged wireworms indicated that liquids do permeate the body wall, the next problem was to find out whether the arsenical salts are carried through with the liquids. Tests for arsenic in various parts of individual wireworms that had been submerged are reported in table 4. In this table it is shown that the quantity of arsenic in the blood increases with the increase in total weight as recorded in table 3. The wash water, on the other hand, does not show any such consistent increase. When the larvae were taken out of the solution, rinsed, and placed on a moist blotter, there was an increase in the quantity of arsenic that could be washed off the integument as time elapsed, some tests showing as much as 5 micrograms. The quantity of arsenic in the fat bodies varied in general

with the quantity of fat and with the length of exposure. The digestive tract, the nerve cord, and the heart showed practically no arsenic, but in the case of the last two this may have been due in part to their small size. The tracheae were consistently free of arsenic. Both the muscles and the integument showed relatively large quantities of arsenic. The muscles usually contained more arsenic than the integument.

DISCUSSION

Contrary to expectation, in the tests with pellets containing arsenic those wireworms that apparently fed did not die. It was noted that certain of the poisoned pellets were markedly repellent to the wireworms. This repellency was found to be more or less in proportion to the solubility of the arsenical present. Nevertheless, the majority of the wireworms that were killed were in the cages with the highly repellent and highly soluble sodium arsenate or sodium arsenite. It was further found that the majority of those that were killed had not attacked the poisoned pellets, and that arsenic was not present in their digestive tracts. In only three cases, or about 1.5 percent of the tests, was arsenic found in the alimentary canal in quantities sufficient to kill. In four other cases the poison was found there, but not in fatal quantities, and the wireworms were still active and appeared normal until they were killed for examination.

Wireworms were repeatedly noted burrowing into arsenically poisoned pellets, with no apparent ill effects. This led to the question as to whether solids are ever eaten by wireworms. Langenbuch (*3*) decided that it was necessary for foods to be in solution before they would be ingested. Repeated observations at this laboratory showed that soil particles were never recognizable in the gastric content, which fact seems to support that view. Lampblack, however, was found in the digestive tracts of several wireworms that had burrowed into pellets containing that material. This showed that solids may be ingested if the particle is small enough. Fine graphite, on the other hand, has a particle size a little larger than that of lampblack, and it was rejected in every case where it was tried.

The Pacific coast wireworm possesses a mechanical means of preventing undesirable substances from entering the digestive tract. In the anterior part of the buccal cavity there are two sclerotic plates which fit tightly together when relaxed, closing off the digestive tube completely. These plates can be separated only by contractions of large muscles which are attached to them and which are controlled by the central nervous system. In addition, there are more than a thousand bristlelike hairs in the preoral cavity, distributed over the anterior edge of the ventral closing plate, on the hypopharynx, and on the mandibles, maxillae, and labial palpi, which act as a filtering device to keep large particles from reaching the digestive tube. This apparatus is probably sufficient to prevent even undesirable soluble materials from entering the digestive tract. The fact that dyes and other nonpoisonous materials were taken in indicates that the larvae have sensoria and can discriminate against the arsenicals. It is concluded, therefore, that the reason arsenically poisoned baits do not kill wireworms is that arsenicals are repellent to them and the larvae do not ingest the poison even when they do come to the baits.

Cornstarch, which has a larger particle size than graphite, was

never recognized in the gastric content. Corn as well as wheat is considered a desirable food for wireworms, frequently being used in rearing experiments. In such experiments the wireworms are commonly found cleaning the starch completely from the seed coat, leaving no pile of rejected refuse. It appears, then, that either very fine grinding or predigestion must take place. The failure of the iodine test to show its characteristic blue color when applied to the gastric content indicates that predigestion must take place and that the starch is reduced at least to achroödextrin before it reaches the stomach. Iodine-stained starch was burrowed into, but the gastric content failed to show either the starch granules or the typical starch-iodine color, which also indicated that predigestion had taken place if the starch was eaten. The tests for reducing sugar were inconclusive. The failure to obtain a positive test did not necessarily mean that digestion beyond achroödextrin had not taken place. The quantity of material for each test was so small that the reduced copper could easily have been overlooked.

Fast green was frequently found in the bodies of wireworms that had access to pellets containing the dye. At times it was found in the wireworms even though the pellets had not been attacked. The capillary action of the fast green through the cellucotton pads gave the suggestion that soluble arsenicals might also move through the pads. Tests showed this to be true, and thus it was seen that sodium arsenite and sodium arsenate were brought in contact with the body of the wireworm even though the pellets were not attacked.

By far the greatest number of wireworms that died in the cages with the poisoned pellets did not have so much as a trace of arsenic in their digestive tracts. Most of them did, however, have large quantities of arsenic in the blood. The arsenic must therefore have entered the body by some means other than by way of the mouth. This finding is contrary to that of Subklew (*9*), who states that salts must enter the body through the digestive tract.

When the wireworms were submerged in arsenical solutions, they took on weight rather rapidly. In the first 6 hours this increase was between 20 and 24 percent, except in the hypertonic 2-percent solution. Tests for arsenic showed that the quantity of arsenic in the blood increased according to the time of submergence. The arsenic in the digestive tract did not increase noticeably, and the concentration of arsenic in the haemocele was constantly and increasingly greater than in the gastric content, showing conclusively that the arsenic did not enter by either the mouth or the anus.

These facts leave only two possible ways in which the arsenic may enter the body, through the tracheae or through the integument. The former is eliminated in the finding of a complete lack of arsenic in the tracheae, and by the observation that these organs were filled with air in all cases where dissections were made. These tubes filled with liquid only after they had been cut and the gases allowed to escape. The integument, then, is the only tissue through which the arsenic could have passed. This is contrary to the generally accepted idea, recently reiterated by Wigglesworth (*10*, *p. 2*), that the integument is impermeable. Mellanby (*6*), too, in his study on site of loss of water from insects, concludes that all the integument is watertight.

Subklew (*9*) admitted that water might pass through the integument but denied that salts could pass through that tissue. The present study shows definitely that at least the sodium salts of arsenic, when in solution, can and do pass through the integument of the wireworm. The finding of arsenic in the body wall supports this conclusion, as does the recent work of O'Kane and Glover (*8*) and Lepesme (*5*).

SUMMARY

This paper reports a series of experiments designed to explain the failure of baits poisoned with arsenicals to attract and kill wireworms. Experiments are also presented that show how and where soluble arsenical salts in solution may enter the body of the wireworm.

In the early experiments the wireworms were confined in metal and glass cages, on moist cellucotton pads with cornstarch pellets. These pellets were made by mixing various arsenicals or nontoxic materials with cornstarch, either with or without the addition of dyes, and casting the moistened mass. The test for arsenic was by the Gutzeit method, but in a modified apparatus reduced to the size that samples of one drop or less could be tested, and an alkaline digestion was used.

Arsenicals were found to be repellent to wireworms more or less in proportion to their solubility. The majority of wireworms that were killed in these experiments were confined with the more repellent materials. Furthermore, most of the wireworms that died had no arsenic in the digestive tract, but had large quantities in the blood. Very firm, solid substances were demonstrated as being ingested, and certain soluble ones were taken in. Arsenicals were rejected, even though the pellets containing them were burrowed into. The sensory control of the closing mechanism in the buccal cavity may account for the rejection of the arsenic.

In the latter part of the study the wireworms were submerged in arsenical solutions and the various tissues tested for the presence of arsenic. Water containing sodium arsenite entered the bodies of the wireworms, carrying the arsenic with it. It did not enter by way of the mouth, anus, or tracheae, but through the integument, which was found to be pervious both to water and to the sodium salts of arsenic.

LITERATURE CITED

(1) Association of Official Agricultural Chemists.
1935. official and tentative methods of analysis . . . Ed. 4, 710 pp., illus. Washington, D. C.

(2) Hawk, Philip B., and Bergeim, Olaf.
1927. practical physiological chemistry . . . Ed. 9, rev. and enl., 931 pp., illus. Philadelphia.

(3) Langenbuch, R.
1932. beiträge zur kenntnis der biologie von agriotes lineatus l. und agriotes obscurus l. Ztschr. Angew. Ent. 19: [278]–300, illus.

(4) Lehman, Russell S.
1933. field experiments with various poison baits against wireworms, limonius (pheletes) canus lec. Jour. Econ. Ent. 26: 243–252.

(5) Lepesme, Pierre.
1937. de l'action externe des arsenicaux sur les insectes. Compt. Rend. Acad. Sci. [Paris] 204: 717–719.

(6) MELLANBY, KENNETH.
1934. THE SITE OF LOSS OF WATER FROM INSECTS. Roy. Soc. [London] Proc., Ser. B, 116: 139–149, illus.
(7) NEW JERSEY AGRICULTURAL EXPERIMENT STATION.
1928. SOIL INFESTING INSECTS, WIRE-WORMS. N. J. Agr. Expt. Sta. Ann. Rept. 49: 172.
(8) O'KANE, W. C., and GLOVER, L. C.
1935. PENETRATION OF ARSENIC INTO INSECTS. STUDIES OF CONTACT INSECTICIDES. X. N. H. Agr. Expt. Sta. Tech. Bull. 63, 8 pp.
(9) SUBKLEW, WERNER.
1934. PHYSIOLOGISCH-EXPERIMENTELLE UNTERSUCHUNGEN AN EINIGEN ELATERIDEN (AGRIOTES OBSCURUS L., AGRIOTES LINEATUS L., CORYMBITES TESSELLATUS L. UND LIMONIUS SPEC.). Ztschr. Morph. u. Ökol. der Tiere 28: [184]–228, illus.
(10) WIGGLESWORTH, V. B.
1934. INSECT PHYSIOLOGY. 134 pp., illus. London.

U. S. GOVERNMENT PRINTING OFFICE: 1938

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 57 WASHINGTON, D. C., AUGUST 15, 1938 No. 4

RATE OF SPREAD OF SURFACE FIRES IN THE PONDEROSA PINE TYPE OF CALIFORNIA[1]

By JOHN R. CURRY, *senior silviculturist*, and WALLACE L. FONS, *assistant engineer*, *California Forest and Range Experiment Station* [2]

INTRODUCTION

Studies of forest-fire behavior have as their broad objective the determination of the natural laws governing primary ignition and subsequent rate of spread of fires in forests and in related types of inflammable vegetation. The purpose of such studies is to provide an improved foundation for fire control, a major activity in American forestry. Scientific evaluation of the factors affecting fire behavior cannot replace experience gained in fire-control operations. Such research can, however, supplement experience by providing information not obtainable on the fire line and, by explaining observed fire phenomena, make them more understandable and predictable. Fire-behavior studies are needed to give information essential to the extension of methods of forecasting fire danger, to permit determination of size-of-crew and speed-of-attack requirements, and, finally, to promote development of fire-suppression tactics and strategy.

Gisborne (*3*),[3] Stickel (*9*), Nichols,[4] and others have studied forest-fire ignition hazard through the determination of variations in fuel-moisture content under varying weather conditions, relating these moisture variations to ignitibility through tests with various firebrands. Through the work of these investigators we know that with ordinary firebrands—as matches, smoking materials, and small campfires—ignition occurs only when the forest fuels contain moisture amounting to less than 30 percent of the dry weight of the fuel. These studies have also resulted in an understanding of the influence of meteorological factors on moisture variations in the fuel, permitting estimation of moisture from a knowledge of weather factors.

Show (*6*) first studied rate of spread in 1916 and 1917, using small test fires. As a result of observations made on 33 fires during this period, he concluded that fires burning under conditions favorable to rapid spread tend to burn with acceleration, while slower-burning fires tend to burn at a steady rate. His observations also led him to conclude that the effect of wind could be expressed as a function of the square of the wind velocity. Through theoretical analyses of the factors influencing combustion in the open, Hawley (*4*) and Gray [5] have contributed in a major way to the advancement of fire-behavior knowledge. Show and Kotok, in analyzing the California fire records, have broadly defined the influence of cover type (*8*) and

[1] Received for publication December 15, 1937; issued August 1938.

[2] Maintained in cooperation with the University of California at Berkeley, Calif.

[3] Italic numbers in parentheses refer to Literature Cited, p. 267.

[4] NICHOLS, L. H. THE BURNING OF SETTLERS' SLASH AND METEOROLOGICAL CONDITIONS IN THE PROVINCE OF QUEBEC DURING 1931. Quebec Forest Indus. Assoc., Ltd. Rept. 4, 22 pp., illus. 1931. [Mimeographed.]

[5] GRAY, L. G. PRELIMINARY REPORT ON FIRE HAZARD RATING STUDY. U. S. Weather Bur., 54 pp., illus. San Francisco. [Mimeographed.]

weather factors (7) on rate of spread. These studies have resulted in determination of the speed of attack required for effective suppression under hazardous conditions.

The present study may be considered a continuation of the work begun by Show in 1916. The primary purpose of both studies was the exploration and definition of the relationships between fuel, fuel moisture, weather factors, and rate of spread. Pure stands of ponderosa pine (*Pinus ponderosa* Dougl.) were chosen for these experiments because it was the simplest fuel type available and because it is an extensive and commercially important timber type. By initiating studies in a simple type, it was considered probable that relationships would be developed of value to the extension of rate-of-speed information to other more complex types.

DETAILS OF STUDY

SELECTION OF VARIABLES

Studies of fire behavior are made complex (1) by the number of factors involved and (2) by the variation found in these factors from place to place and from time to time within the forest. Wind normally fluctuates widely both in intensity and direction, and this characteristic is intensified in the forest by the resistance offered by the foliage and tree trunks. Fuels in themselves vary widely from place to place within the stand, and the moisture content of fuels is constantly changing with changes in weather conditions.

Six primary factors are recognized in rate-of-spread studies, viz: (1) Fuel size and arrangement; (2) moisture content of the fuel; (3) air supply (wind movement); (4) slope of the ground; (5) the tendency of fires to burn at an increasing rate, or the effect of time; and (6) size of the fire. The first five factors listed are independent variables while the sixth is the variable under study, or the dependent variable. Although other important variables may be defined by further research as affecting fire spread, the problem may at this stage be considered essentially one of relating rate of increase in fire size to concurrent variations in the five other variables listed.

Present knowledge of the factors influencing combustion in the open contains no information on methods of measuring one factor, variation in the physical characteristics of forest fuels. Hawley (4) has suggested the use of the surface-volume ratio as an approach, but the specific technique of measuring this, or any other fuel factor which might be used to explain rate-of-spread fluctuations, remains to be developed. This lack of a well-defined theory and technique for the measurement of the fuel variable made it necessary in the present study to eliminate this factor so far as possible. This was the primary reason for the selection of the ponderosa pine type, which contains the most uniform fuel conditions to be found in California. Under some situations in this type, usually in dense even-aged stands, the fuels present consist almost entirely of a layer of pine needles from 1 to 4 inches in thickness.

SELECTION OF SITES

Characteristic virgin stands of ponderosa pine were not suitable as sites for this study. They consist of groups of mature and overmature trees interspersed with more open areas occupied with brush,

or reproduction of various ages. Litter accumulates to depths of 4 to 5 inches within the dense groups, while a few feet away, in an opening between groups, the ground may be bare. Such fuel conditions were too uneven to meet the study specifications.

Only two kinds of stand within the type were found with sufficiently uniform surface fuel. Optimum conditions were found within certain even-aged dense stands of ponderosa pine on the Shasta Experimental Forest, usually 45 to 50 years of age, and averaging about 600 trees to the acre, with an average diameter breast high of 9 inches, and a height of 60 feet. Certain uniform, relatively dense even-aged virgin stands in which the younger trees had been killed by light fires were also well adapted to the study. The generalizations made in this report as to surface fires in the ponderosa pine type should therefore be applied specifically only where the fuel conditions are uniform; that is, where the ground is completely covered by at least 1 inch of pine needles.

Fortunately for the simplification of the first year's study, the second-growth stands on the Shasta Experimental Forest occurred on level ground, permitting the elimination of slope as a variable and reducing the number of variables to be measured to four, viz: (1) Size of fire; (2) moisture of fuel; (3) wind velocity; and (4) effect of time. In all, tests were conducted on 13 carefully selected sites during the first year's study. During 1934, the fires were confined to two sites and included studies of fires on sloping ground.

CONDUCT OF TESTS

The primary factors measured on each fire were fuel moisture, wind velocity, perimeter at each 2-minute interval, and, where important, slope. In addition, several secondary factors were recorded, as depth of litter, air temperature, surface-fuel temperature, relative humidity, and barometric pressure.

For the plotting of perimeter, steel stakes were set at 5-foot intervals on lines radiating from the central ignition point. With these stakes in place it was practical for the plotter to enter the perimeter of the fires directly on polar coordinate paper. Figure 1 illustrates a fire 8 minutes after ignition on a typical test plot. Plotting was ordinarily done on a sketching tripod, as shown.

Samples for moisture-content determinations were gathered immediately before the start of the fire. Two methods were used at different times during the tests. The first consisted of gathering two samples, one in an open exposed part of the test plot, and a second in a more dense, covered portion. By the second method a single sample was taken of the top needles over the whole plot. Because rather wide variations were found over the plots, the average moisture value obtained by the second method proved the most useful. Samples were collected in friction-lid cans and were weighed the day of collection. Because of lack of facilities in the field, all moisture determinations were made at one time, at the end of the field season. The initial moisture determinations were based on ordinary oven-drying procedure. Later, studies by Buck (*1*) led to the use of a more accurate and speedy xylene distillation method. Moisture percentages used in this report have all been reduced to the xylene

distillation standard, which is about 1.5 percent higher in indicated moisture content than originally computed on an oven-dry basis.

The measurement of wind velocities on the test fires presented problems in the selection of an instrument and in the choice of height above ground surface at which the measurement would best represent the influence of wind on rate of spread. Wind velocities within closed stands are relatively light in comparison with concurrent velocities in the open, but nevertheless have a great influence on rate of spread. The ordinary three-cup anemometer was found to lack the sensitivity required for measuring these light winds. The Biram type (fig. 2) is much more sensitive and was used on all fires studied. To adapt this anemometer to the study of test fires, it was mounted on bearings, and provided with a vane to maintain its axis parallel to the wind



FIGURE 1.—Test fire in typical even-aged stand of ponderosa pine 8 minutes after ignition. The uniform nature of the fuel is characteristic of the locations sought in these experiments.

direction. So adapted, this type is recommended for all similar studies. An anemometer on the hot-wire principle was tried later, but produced records no more useful than those obtained with the Biram type. After some experimentation, 30 inches above ground surface was selected as the desirable standard height for measurement of wind velocities, and throughout the study the anemometers were so mounted. Although an automatic recorder was developed toward the end of the experiment, the data used in the analysis consisted entirely of velocities recorded visually each 30-second interval over the period of the tests.

Time records were taken with stop watches from the instant the match was applied. On a portion of the fires, temperatures attained by the fire during combustion were measured with thermocouples. Records of air temperatures, litter temperature, relative humidity, and barometric pressure were obtained with standard instruments. A detailed description, including needle depth, crown density, open-

ings in the canopy, and size of trees, was made for each plot. A running description of the behavior of each fire was a part of the routine observations.

On the smaller fires, one man plotted perimeter, a second recorded wind movement, while a third made a running description of the fire. On the larger fires the number of men was increased to a maximum of seven, to allow more men for recording wind velocity, plotting perimeter, and operating the thermocouple apparatus.

In the test-fire procedure, after the selection of the test plot by the chief of party, a crew set the lines of stakes, using compass and tape, and taking care to disturb as little as possible the natural condition of the surface litter. While this work was proceeding another crew removed from the plot all large limbs and other debris which might influence the spread, and trimmed low dead branches which might tend to make the fire crown. In the early experiments, fire lines were cleaned around the test plots, but this practice was later abandoned, since the lines were seldom used, their preparation required considerable time, and their use reduced the number of plots that could be studied on any one site.

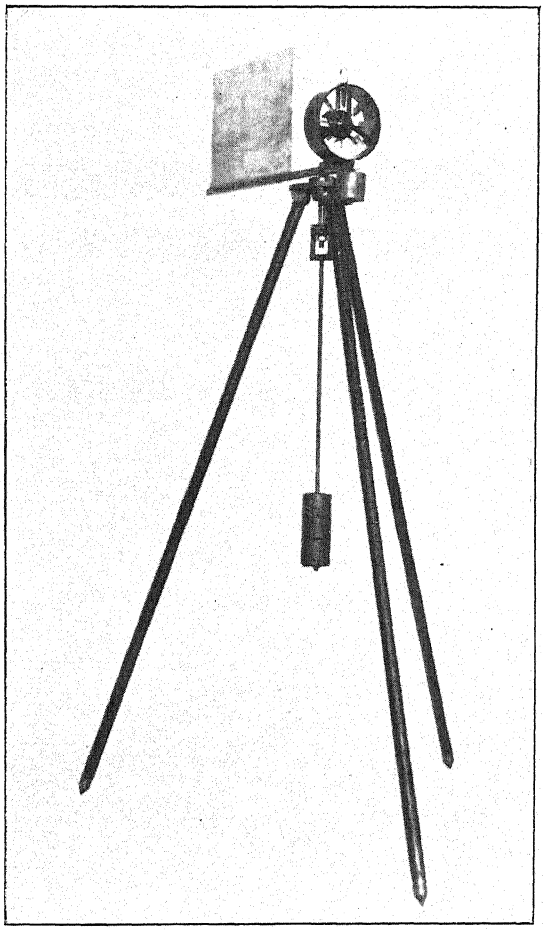


FIGURE 2.—Biram (vane) type anemometer mounted on a special tripod and fitted with a large direction vane.

During the process of plot preparation, the plot description was made, emphasizing any abnormal or unusual features; record forms were prepared; and the anemometer was set up to windward within 10 feet of the estimated reach of the fire line. Thermometers were exposed in the litter, to be read just prior to the start of the fire.

Fire-fighting equipment was then assembled, water supplies inspected, and the suppression crew distributed. At this point a check on the progress of all preparations made it possible to set a time for the start of the fire. The final records, temperature, humidity, and

litter moisture were taken within a 5-minute period preceding the starting time.

The fire was started by applying a single match at the center stake and simultaneously the stop watches of the observers were started. Thereafter all records of wind, perimeter, and fire behavior were maintained according to stop-watch readings. Perimeter was recorded on the smaller fires at each even 2-minute period. On larger fires it was found desirable for the plotter to travel continuously around the fire, marking the perimeter location and the time of observation to the second. The accumulated total wind movement was read at each 30-second interval.

At the conclusion of the test, the fires were stopped by the application of water. With the edge of the fire dead, the smoldering litter was systematically extinguished to the center. By fast, thorough action it was usually possible to extinguish a test fire completely and to clear a fire line to mineral soil around it within 20 minutes of the end of the study. With experience, it was possible to obtain from two to five tests a day, depending on the area of the fires. Test plots were inspected regularly for several days following the fire.

During the 1933 field season, 95 selected test fires were studied on the Shasta Experimental Forest, under uniform conditions in the ponderosa pine type. During this season, the fires were allowed to attain a size of one-sixtieth to one-fortieth of an acre, the maximum size readily extinguishable with the facilities available. The period of study was ordinarily 18 minutes, although some slow-burning fires were allowed to spread for 30 minutes or more. Extremely rapid-burning fires were, for reasons of safety, suppressed at 12 or 14 minutes.

Preliminary analysis of the 1933 records showed that the data could be much improved through additional studies over a longer total burning period. Some data on the effect of slope were also desired. In 1934 an area in ponderosa pine was selected on the Lassen National Forest, 100 miles south of the 1933 center of work, and here 69 fires were set, 46 of these being on slopes above 10 percent in grade. During this second season, the tests ran for 30 minutes on the 23 fires on level ground. These fires attained areas of 0.05 to 0.5 acre. The suppression problem was increased to an extent sufficient to reduce materially the number of fires studied.

GENERAL CHARACTERISTICS OF THE DATA

A cursory examination of the data available at the completion of the tests revealed large fluctuations in rate of spread at different hours of the day. A fire at 7 a. m. might reach a perimeter of 25 feet in a 12-minute period and later, on the same day, another might attain 150 feet during the same burning period. Even average values fluctuated widely with time of day, as shown in table 1.

The summer climate of northeastern California is remarkably uniform. During the period of these tests, little or no precipitation occurred and clouds were uncommon. Minimum temperatures at night ranged from 30° to 55° F., while maximum day temperatures were within a range of 70° to 90°. Relative humidities in the summer at this elevation (4,000–5,000 feet) vary from 100 percent in the early morning hours to a minimum of 10 to 11 percent in the early afternoon.

TABLE 1.—*Hourly averages of rate-of-spread factors of fires, 1933–34*

Hour of day	Fires	Area in 12 minutes	Perimeter in 12 minutes	Wind velocity per hour	Moisture content	Relative humidity	Air temperature
A. M.:	*Number*	*Square feet*	*Feet*	*Miles*	*Percent*	*Percent*	*° F.*
7	1	60	30	0.0	12.5	45.0	72.0
8	6	95	36	.3	10.0	60.8	58.7
9	12	164	44	.9	9.9	42.9	66.9
10	21	285	62	2.1	9.1	31.8	73.7
11	14	320	66	1.7	8.6	28.4	75.5
12	2	295	64	1.2	7.6	22.5	83.0
P. M.:							
1	14	476	84	2.0	7.0	22.2	83.2
2	17	458	80	2.0	6.6	22.9	81.4
3	11	328	67	1.3	7.1	22.8	81.9
4	11	257	60	1.1	6.8	23.3	82.5
5	3	212	54	.6	6.9	31.7	78.3
6	2	104	38	.0	8.0	48.5	68.0
7	1	133	42	.5	8.3	53.0	68.0
8	2	166	48	.8	9.0	54.5	66.5

One striking fact illustrated by table 1 is the average low wind velocity recorded on these fires. From this it might be inferred that the tests were conducted purposely at times of light wind. In fact, however, windy conditions were sought rather than otherwise, but velocities within the stands were seldom greater than 3.5 miles per hour and then only for short periods. This was due to high efficiency of the timber as a windbreak. It can be stated as a result of these observations, and on the strength of further studies by the authors, that winds near the ground in moderately dense stands seldom attain a velocity greater than 3 miles an hour even when in the open they reach 30 or more miles an hour.

The tendency illustrated in the table for high wind velocity and low moisture content to occur during the same periods was noted in conducting the tests. Wind in the area where the tests were located is largely controlled by thermal convection, with the strongest movement during that part of the day when air temperature is highest. Absolute humidity tending to remain constant, the moisture content of light fuels in the areas studied responded quickly to changes in temperature. While this phenomenon is undoubtedly characteristic of other forests, it is particularly pronounced in the summer climate of interior California where air conditions are characteristically stable.

The average moisture content of litter shown in table 1 ranges from 6.6 to 12.5. The total range on these plots was from 4.0 to 13.5 percent. Since all fires were studied during a period when rain had not fallen for several weeks, it may be assumed that these variations are the result of diurnal fluctuations in atmospheric conditions.

Figure 3 illustrates characteristic types of perimeter records. The lines on the chart represent the margin between flames and unburned litter at successive 2-minute intervals. On the slower fires the width of the flaming area was about 6 inches, but on fast fires the flames might attain on the leeward side a width of several feet.

The simplest type of fire is that shown in figure 3, *A*, where air movement is too light to affect spread appreciably. Such a fire soon develops into a thin ring of fire with the flames turning inward and upward as shown in figure 1. This tendency of flames to burn inward

is due to the displacement of the hot gases rising from the smoldering interior by cool air from outside the ring of fire. In the absence of changes in fuel conditions, fuel moisture, and wind, the rate of spread of such a fire would vary only during the period when the fire was becoming established, and with changes in the indraft due to variations in the volume of rising gases. The regularity shown in the burning rates of fires of this type gave support to the assumption of uniform fuel conditions.

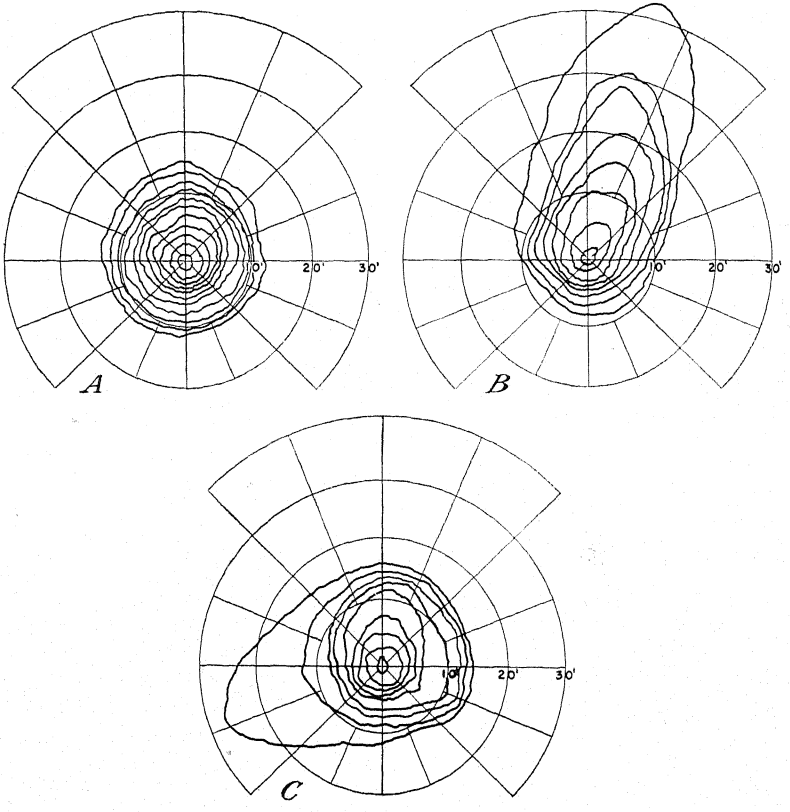


FIGURE 3.—*A*, Diagram of slowly spreading fire: Average wind velocity 0.2 mile per hour, fuel moisture content, 7.0 percent; perimeter shown at each 2-minute interval. *B*, Diagram of rapidly spreading fire with constant wind direction: Average wind velocity 2.0 miles per hour; fuel-moisture content 4.3 percent; perimeter shown at each 2-minute interval. *C*, Diagram of rapidly spreading fire with variable wind direction: Average wind velocity 1.1 miles per hour; fuel moisture-content 9.0 percent; perimeter shown at each 2-minute interval.

The chart of a fire burning under a wind of constant direction and under comparatively uniform velocity is shown in figure 3, *B*. Although fires starting under these conditions are at first not greatly affected by the wind, they spread much more rapidly after becoming established. The shape of the fire and the rate of spread are in this case very noticeably affected by a wind of an average velocity of 2 miles per hour.

With a wind of more than 1 mile per hour, the influence on the size and shape of the flames is immediately noticeable. Flames on the

leeward side increase in size and turn outward, becoming with higher velocities approximately parallel with the surface of the ground. On the windward side, the flames are reduced in intensity and driven toward the burned area. On the flames burning at right angles to its direction, the wind has apparently little effect.

Figure 3, *C* illustrates a type of fire more characteristic of normal conditions than those shown in the two preceding charts. In this case the wind movement varied both in rate and direction. Fires behaving in this manner increase their area more rapidly than fires burning with a comparable velocity in one direction only. The effect on perimeter of such variation is, however, unimportant.

METHOD OF ANALYSIS

The first step in the analysis of the fire records consisted of measuring the perimeter increase from charts such as those shown in figure 3. Perimeter was obtained with a map measurer and checked to the nearest foot. These data, together with the corresponding data for wind velocity, moisture content, and time for the 118 fires on level ground, were then entered on 1,153 cards, each containing the complete record of one 2-minute interval. Because of the scarcity of data in the higher time classes, all cases above 22 minutes were eliminated. The reason that perimeter and not area was used is that the area of a circle, or an ellipse, increases in proportion to the square of its radius, while the perimeter increases in direct proportion to the radial increase. The rate at which the area of a fire increases is, consequently a function of the size of the fire, while the rate of perimeter increase is, geometrically speaking, independent of size. Furthermore, perimeter is of greater significance in fire control, as better representing the suppression effort required.

The Pearsonian mathematical method of multiple linear correlation was first considered. Two difficulties presented themselves, however: (1) The relationships so derived would be linear, whereas it was considered probable that the relationships were in part curvilinear; (2) this form of analysis is unsuited to definition of relationships where joint correlation exists; that is, where the influence of any one independent variable on the dependent variable is affected by the size of another independent variable. Show (*6*) discovered the presence of joint correlation in his 1916 data and preliminary curves in the present analysis confirmed this finding. Consequently, methods which would permit the definition of joint correlation, if it existed, were desirable. These considerations led to the use of methods recommended by Ezekiel (*2*) for problems of joint correlation. Inasmuch as these methods enter into discussion of the analyses finally developed, they are explained in detail.

First, all cards were sorted into 2-minute time groups. Next, the cards in each time class were sorted into three moisture classes—below 7.4 percent, 7.5–10.4, and 10.5 and more. A third segregation was then made within each moisture class, on wind velocity, five wind-velocity classes being recognized as follows: 0.0–0.9 miles per hour, 1.0–1.9, 2.0–2.9, 3.0–3.9, and 4.0+. Eleven tables of the type shown in table 2 resulted, one for each 2-minute period.

TABLE 2.—*Summary of values of wind velocity, moisture content, and perimeter for first 2-minute periods,*[1] *on a basis of 118 fires on level ground*

Class value X_2	N	Total X_1	Mean X_1	Total X_2	Mean X_2	Total X_3	Mean X_3
FOR CLASS VALUE X_3=0.0–7.4							
0.0–0.9	12	94	7.8	8.7	0.7	79.2	6.6
1.0–1.9	23	203	8.8	31.7	1.4	147.2	6.4
2.0–2.9	5	43	8.6	11.5	2.3	29.0	5.8
3.0–3.9	4	44	11.0	14.3	3.6	22.8	5.7
4.0+	4	42	10.5	19.0	4.8	25.2	6.3
FOR CLASS VALUE X_3=7.5–10.4							
0.0–0.9	23	147	6.4	9.3	0.4	202.4	8.8
1.0–1.9	20	137	6.8	26.7	1.3	182.0	9.1
2.0–2.9	11	81	7.4	26.8	2.4	93.5	8.5
3.0–3.9	1	8	8.0	3.2	3.2	7.8	7.8
4.0+	3	25	8.3	13.1	4.4	26.1	8.7
FOR CLASS VALUE X_3=10.5+							
0.0–0.9	9	46	5.1	1.5	0.2	99.0	11.0
1.0–1.9	2	9	4.5	2.3	1.2	21.6	10.8
2.0–2.9							
3.0–3.9	1	5	5.0	3.3	3.3	11.0	11.0
4.0+							

[1] X_1=Perimeter increase in feet per 2 minutes; X_2=wind velocity in miles per hour, average during 2-minute period; X_3=moisture content in percent, based on xylene distillation method; N=number of cases.

The next step was to plot the average value of the dependent variable (perimeter increase) on the average values of wind as a first approximation of the influence of that variable. Wind was used in the first approximation because preliminary studies had shown its influence to be most strongly defined. Three curves showing the effect of wind on perimeter increase, one for each moisture class, were constructed freehand for each time interval, a total of 33 curves in all. These curves served as a first approximation of the influence of wind velocity on perimeter increase throughout the entire range of moisture and time conditions.

To determine the influence of moisture, a second series of approximation curves was drawn, using the first curves as a base, with moisture content plotted on the horizontal axis. After these plotted points were smoothed by freehand, a third approximation was made with time plotted on the horizontal axis. The fourth smoothing was made with wind velocity plotted on the horizontal axis as in the first approximation. Changes in the relationships became smaller at each smoothing and were finally considered negligible after the seventh and final smoothing.

Several correlation studies of this type were made from the data. The results obtained are dependent to some degree upon the first approximation, where the personal judgment of the one analyzing the data is important. Checks on the final results were made by smoothing in one correlation first on wind and in another first on moisture. Although the variations obtained in the final curves were not great, it was desirable to find a method of analysis less subject to this type of variation.

As anticipated, joint correlation existed in the data and was particularly noticeable in the change in the slope of the curves of wind

velocity with increasing time. Some changes were also noted in the slope of the moisture curves with variations in time. On the other hand, no tendency for the slope of the moisture-perimeter curves to vary with changes in wind velocity, or vice versa, was found in any of the several correlation studies made. This and the fact that the influence of both wind and moisture were established by these studies as linear led to a slightly different type of analysis.

If joint correlation was not present between the wind and moisture factors, but only between time and these factors, it was apparent that multiple linear regressions could be obtained for each time interval, thus eliminating personal judgment in the preliminary curving process. The influence of time, a relationship known to be curvilinear, could then be determined by successive freehand smoothings. This process was followed as outlined, multiple linear regression equations being obtained for the influence of wind and moisture for each 2-minute interval.

At this stage of the analysis a further check was obtained on the presence of curvilinearity in the moisture-perimeter and wind-perimeter relationships. Using the methods outlined by Mills (*5*), it was determined that no significant tendencies toward curvilinearity existed in any of the 11 correlations.

The next step in the analysis consisted in smoothing the regressions to obtain an expression of the influence of time. This was accomplished in the same manner as has been outlined in the discussion of the preliminary analyses. As a result of this procedure, a higher index of correlation and lower standard error were obtained than where freehand smoothing methods only were used. After the curves had been defined by successive smoothings an empirical formula was derived to express the curves mathematically. This formula and the method of derivation will be discussed subsequently. The analysis of the influences of slope on rate of spread, which was handled in a somewhat different manner, will also be discussed later.

GENERAL RESULTS

As an introduction to the results of the analysis, it may be well to review the theoretical aspects of combustion of wood as summarized by Hawley (*4*). The ignition temperature of dry wood is approximately 540° F. Inasmuch as the temperature of combustion is in the neighborhood of 1,500°, it is apparent that combustion of finely divided wood substance will readily proceed, once ignition is established, even in the presence of appreciable amounts of moisture in the fuel. The influence of moisture is explained by the fact that before combustion of wood can take place, all water in it must be raised to 212°, vaporized, and the vapor raised to the ignition temperature of wood, 540°. Theoretically the amount of heat produced permits combustion to the point where 5.71 parts of water are present to 1 part of wood, assuming full utilization of all heat produced in the vaporization of the moisture. From a practical standpoint, however, because of heat loss, wood will not burn even under forced draft if there are more than 2 parts of water to 1 part of wood. Under forest conditions, only a small proportion of the total heat of combustion will be available for this purpose. Any considerable moisture content in forest fuels thus slows down combustion, through decreasing the

According to Hawley, at least 133 cubic feet of air are required to burn 1 pound of wood. This explains in part the influence of wind upon combustion. Wind also influences surface fires by forcing the flames close to the ground surface, thereby increasing radiation to the fuels.

It is common experience that once a fire is established it tends to burn more readily. It has been generally assumed that forest fires rapidly increase their rate of spread with time. In this investigation,

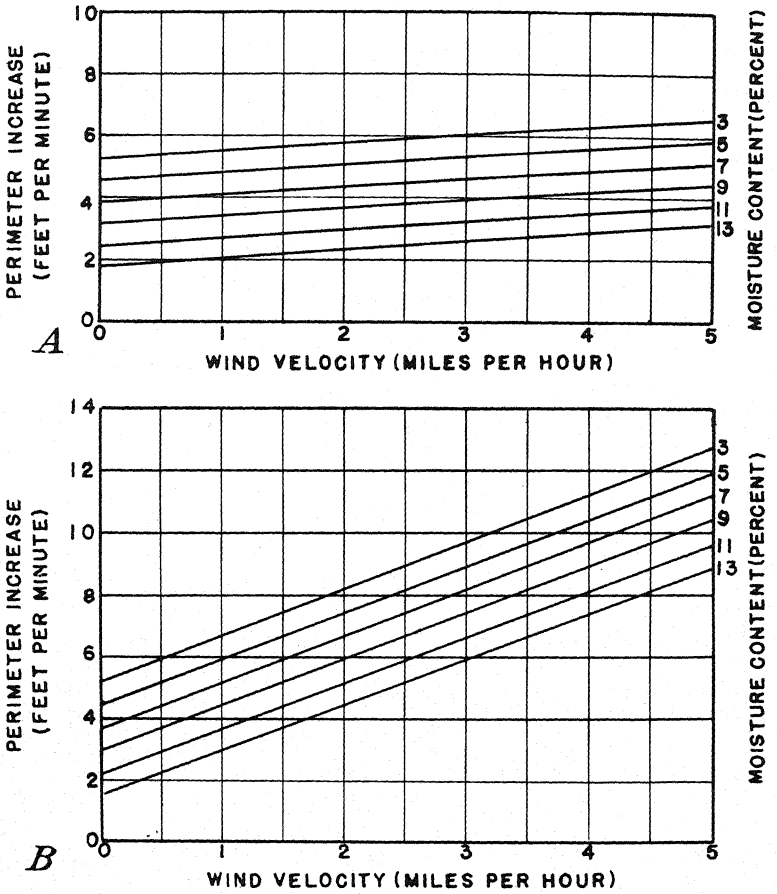


FIGURE 4.—Probable rate of perimeter increase on level ground for various wind velocities and moisture content at 2 (*A*) and at 8 (*B*) minutes from start of fire.

an attempt was made to evaluate the length of this establishment period.

In the following discussion, the effects of wind, moisture, and time are treated separately, prior to a discussion of their combined effect.

THE EFFECT OF WIND

Smoothed curves showing the effect of wind throughout the ranges of moisture and wind velocity are shown in figures 4 and 5. The representation of the influence of wind as linear is in disagreement

with the conclusions of Show (*6*). These earlier conclusions are apparently explained by the fact that it was impossible, with the limited data available in the earlier study, to separate the influence of moisture from the influence of wind. The presence of intercorrela-

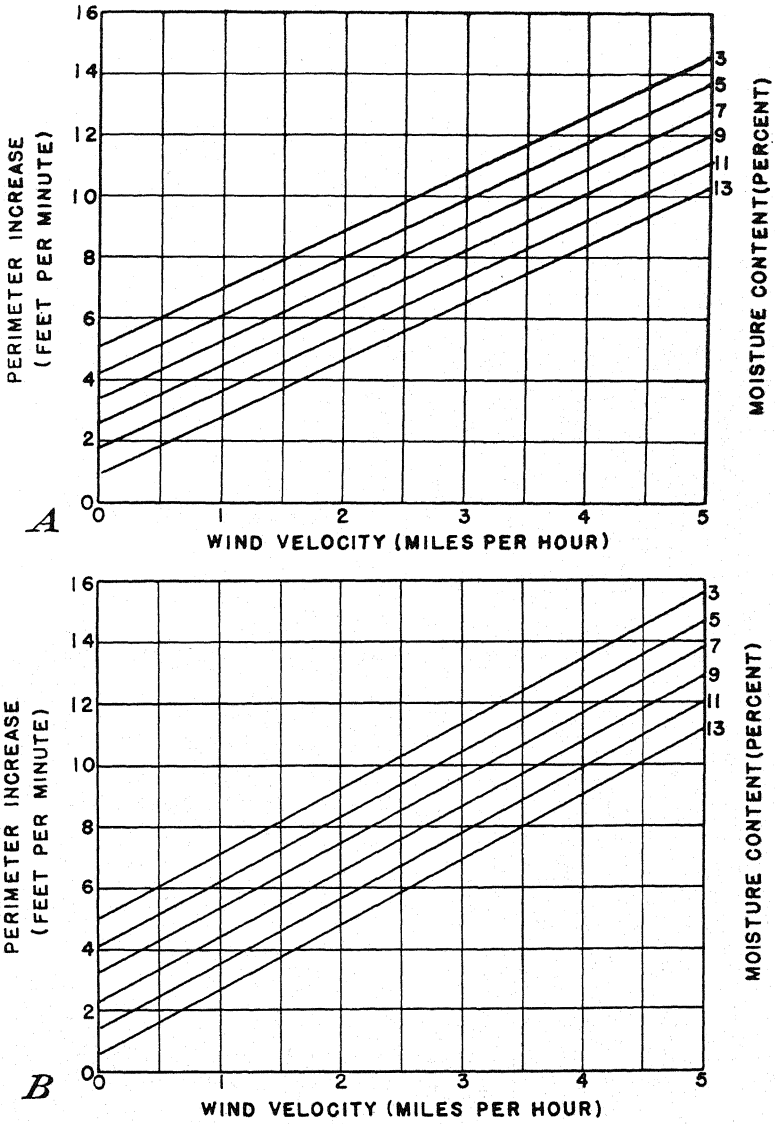


FIGURE 5.—Probable rate of perimeter increase on level ground for various wind velocities and moisture content at 14 (*A*) and at 20 (*B*) minutes from start of fire.

tion between wind and moisture was apparently not taken into account in this early work. In the present analysis, it has been possible to consider the influence of each variable within a small range of variation of the other variables.

A second characteristic of these curves deserving comment is the change of slope with increase in time. At 2 minutes, for instance, rate of spread increases about 0.3 foot with an increase of 1 mile per hour in wind velocity; at 20 minutes the increase is slightly more than 2 feet. The fact that joint correlation does not exist between moisture and wind is shown by the same slope of the lines for all moisture classes.

THE EFFECT OF MOISTURE

The influence of variations in moisture content of the litter is shown in figure 6. As in the case of wind velocity, the relationship shown

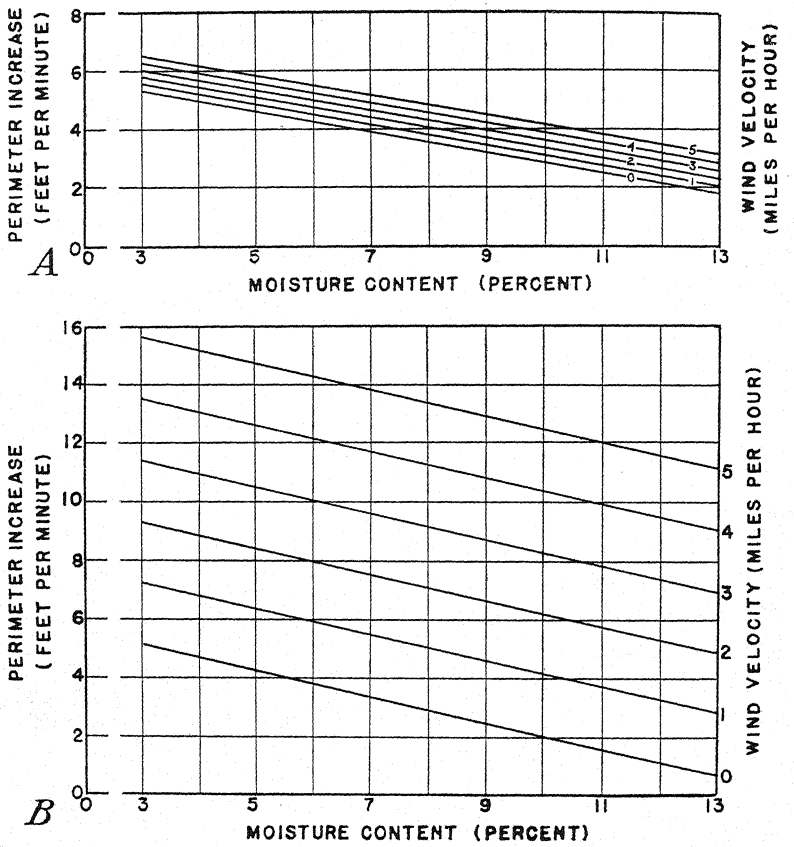


FIGURE 6.—Probable rate of perimeter increase on level ground for various values of litter moisture content and various wind velocities at 2 (*A*) and at 20 minutes (*B*).

is linear throughout the range of time since start of fire; the slope of the moisture curves is negative. Only sufficient curves are shown to illustrate the general character of the moisture-perimeter relationship.

Joint correlation between time and moisture is shown by the variation in the slope of the moisture curves with changes in time; the variations, however, are less pronounced than in the case of the perimeter-wind curves. Because of this joint correlation, it is difficult to generalize broadly on the influence of either the wind or moisture. If any one time is selected, a relative comparison of the influence of

wind and moisture content is possible. At the 20-minute time interval the influence of 1 percent variation in moisture is approximately 0.4 foot in perimeter (fig. 6, *B*). At the same period, the influence of

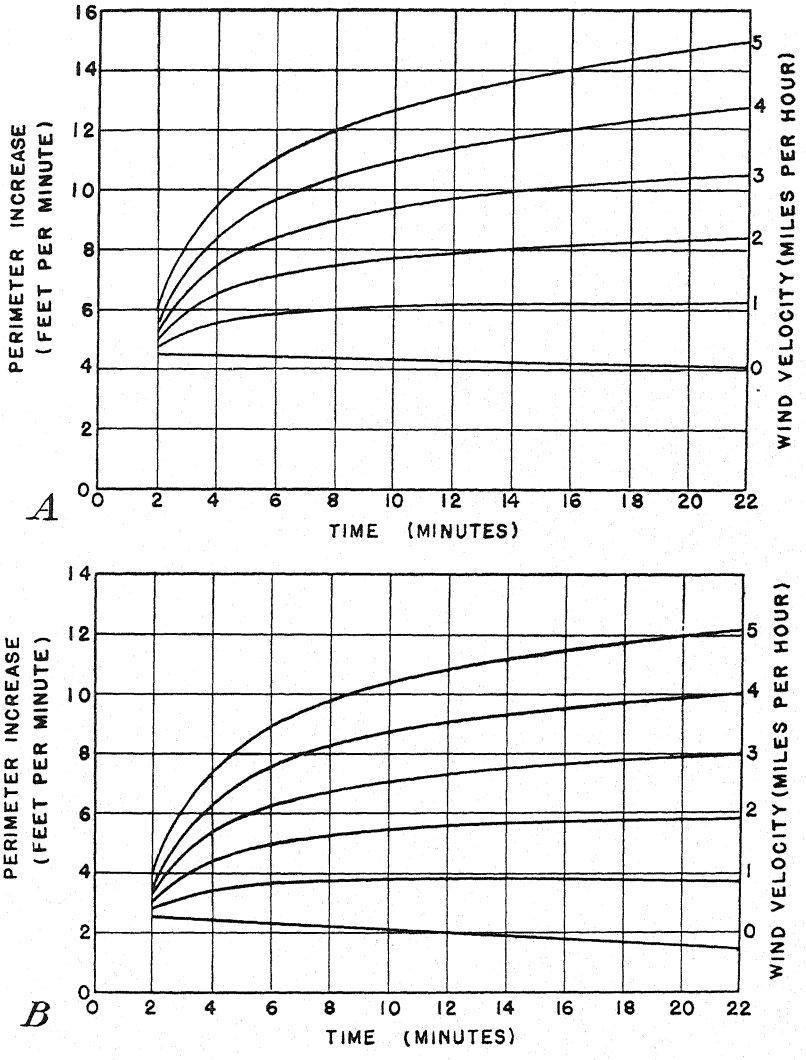


FIGURE 7.—Probable rate of perimeter increase on level ground at various times from start of fire and at various wind velocities for litter moisture content of 5 (*A*) and of 11 percent (*B*).

1 mile per hour in wind velocity is 2.0 feet (fig. 5, *B*). This ratio of 1 to 5 emphasizes the importance, as defined in this study, of wind on rate of spread, once a fire is started. In making this or similar comparisons, it should be remembered that the wind velocities shown are those measured within the stand, and do not correspond to those measured in the open. A relatively large increase of wind velocity in the open would be required to increase velocity within the stand from 3 to 4 miles per hour.

THE EFFECT OF TIME

The effect of time on rate of spread, or, as it might be expressed, the tendency of small fires to accelerate their rate of spread under various moisture and wind conditions, is illustrated in figure 7. Curves are shown for two moisture classes only—5 and 11 percent. It will be noted that, under low wind, fires tend to assume a constant rate of perimeter increase after the first few minutes of burning. With higher winds, the length of the period over which acceleration occurs increases and at the higher velocities the increase is still appreciable at 22 minutes. Presumably, however, under constant wind and moisture conditions, surface fires of this type would tend at the end of one-half hour or slightly more to burn at a steady or only slightly increasing rate.

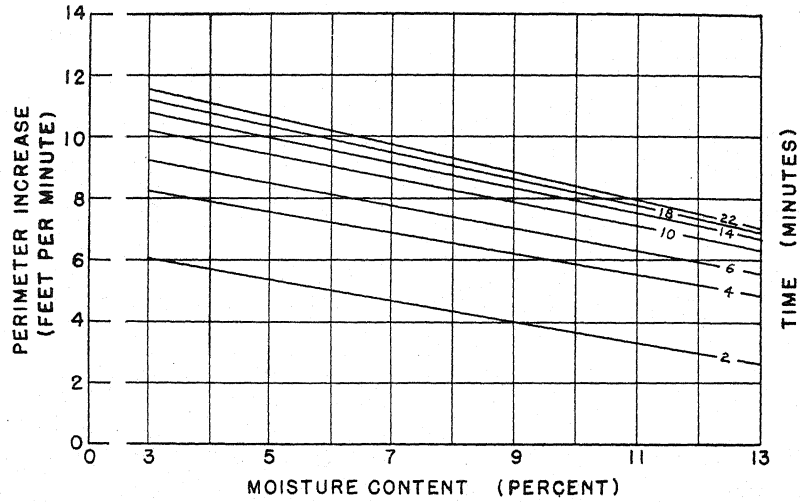


FIGURE 8.—Probable rate of perimeter increase on level ground for various values of litter moisture content and time from start of fire with wind velocity at 3 miles per hour.

COMBINED INFLUENCE OF WIND, MOISTURE, AND TIME

Consideration of the manner in which the three variables—wind, moisture, and time—act in combination to produce various rates of spread is made complex by the presence of joint correlation between time and the other independent variables. In general, it may be said that the influence of both moisture content and wind are direct, i. e., linear; and that a variation of 1 percent in moisture will influence the rate of spread by 0.4 foot per minute, while a change of wind velocity of 1 mile per hour will result in a variation in perimeter of about 2 feet per minute. These values are attained, however, only after the fire has burned for some time and they are not constant but vary with the period during which the fire has been burning. Apparently fires tend to reach a constant perimeter increase under unchanging moisture and wind conditions, but the time when this occurs cannot be closely defined from this study. It would appear, however, that the effect of time would be removed in most cases after 30 to 45 minutes. Figures 8 and 9 have been constructed to

show the variation in the effect of wind and moisture with time for the span of these tests.

Figure 10 is a drawing of a model constructed to show these relationships simultaneously. In this model, lines cutting through the diagram define the rate of spread values for various values of wind and moisture at a given time period.

The combined influence of wind, moisture, and time on rate of perimeter increase may be more definitely expressed in mathematical terms. From figures 4, A, and 6, A, and 4, B, and 6, B, it is obvious that the relationships between perimeter, wind, and moisture for any

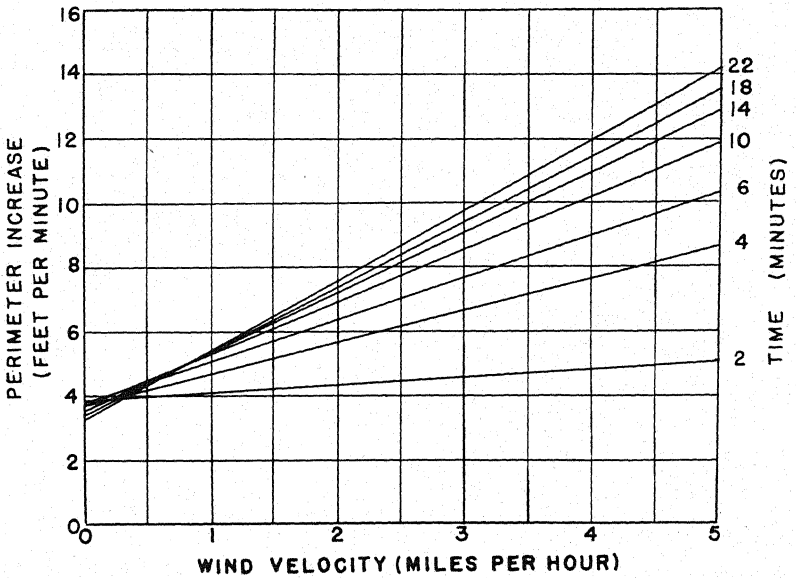


FIGURE 9.—Probable rate of perimeter increase on level ground at various times from start of fire and at various wind velocities, for litter moisture content of 7 percent.

particular time interval, as 2 minutes, may be expressed by a linear equation of the form

$$p=k-k' M+k'' V \qquad (1)$$

in which

p=rate of perimeter increase in feet per minute
V=wind velocity in miles per hour
M=moisture content in percent

The use of the minus sign with the moisture factor is explained by the slope of the moisture-perimeter curve. If formulas are derived for each time period it will be found that the constants k' and k'' will vary as some function of time. If they could be expressed mathematically and introduced into the formula, a mathematical expression of rate of perimeter increase at any time would result.

To do this, values of k' for various times from the smoothed curves were plotted on time. The variation in k' with time is small; in theory it should become constant after a short period. Within the limit of 22 minutes, however, it is practical to treat the relationship as linear,

as shown in figure 11, *A*. An expression for k' in terms of t is readily obtainable by a linear equation in the form of $k'=a+bt$.

When the values of k'' are plotted on time as in figure 11, *B*, the curve resulting is of a more complex type. This may be expressed as a power series of the form

$$k''=a+bt+ct^2+dt^3+---mt^n$$

but would have such a large number of terms that its use would be cumbersome.

The value of the constant k may be determined by extrapolation of the smoothed curves to determine the value of p when both M and

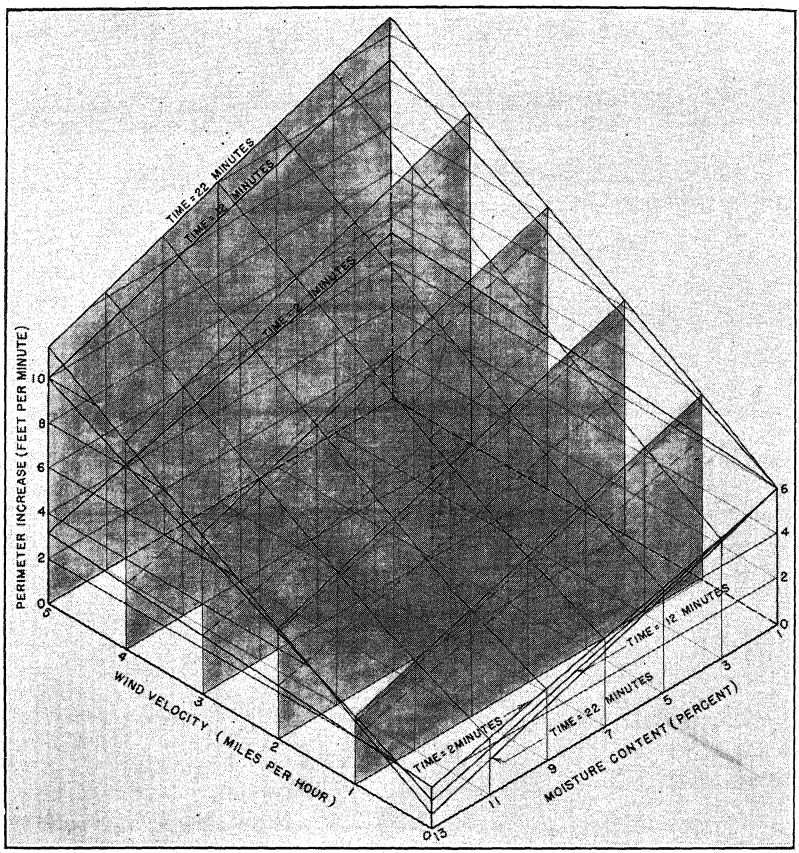


FIGURE 10.—Chart showing relation between wind velocity, moisture content, time from start, and probable rate of perimeter increase, for surface fires in ponderosa pine needles on level ground.

V are zero. This value, so derived, is 6.35, a constant for any time. The final equation is:

$$p_t=6.35-k'\,M+k''\,V \qquad (2)$$

in which

p=rate of increase in perimeter in feet per minute
t=time in minutes from start of fire
k' and k''=constants determined from table 3
V=wind velocity in miles per hour
M=moisture content percent

THE EFFECT OF SLOPE

Inasmuch as forest topography is usually mountainous, and slope is recognized as an important factor in spread, this discussion would be incomplete without some consideration of the slope factor, which has been omitted up to this point mainly to simplify the problem. Studies of slope were conducted under both laboratory and field condi-

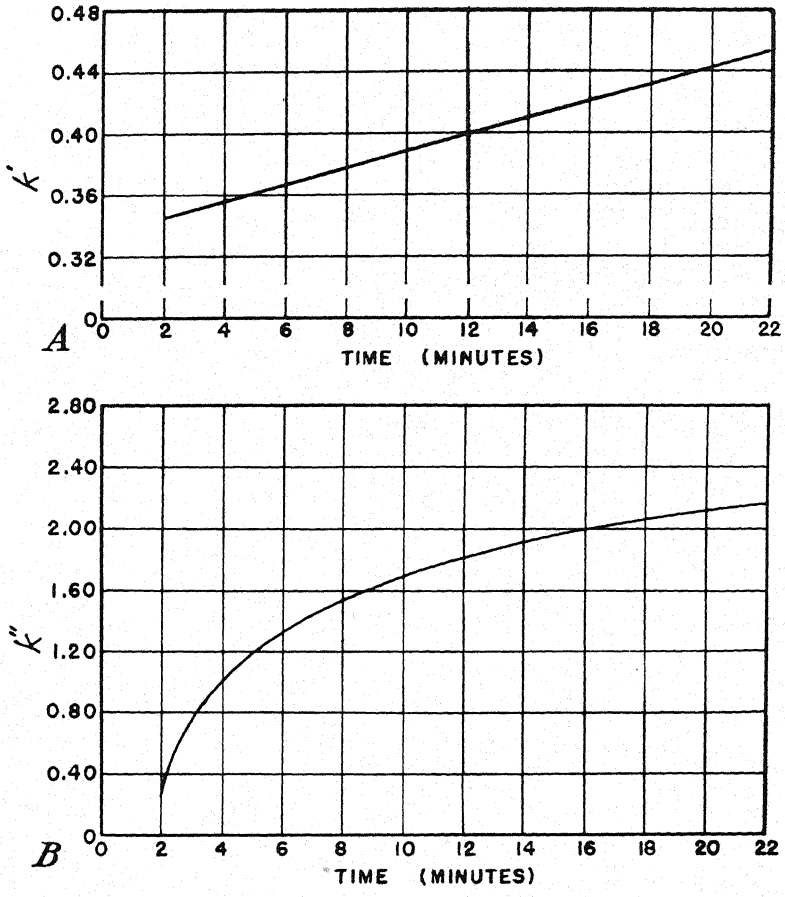


FIGURE 11.—*A*, effect of time on constant k' in equation $p=6.35-k' M+k'' V+f(SV)$. *B*, effect of time on constant k'' in equation $p=6.35-k' M+k'' V+f(SV)$.

tions. Field studies were in all respects similar to those described for fires on level ground.

In the study in the forest fire laboratory of the University of California a series of small fires were allowed to spread in a 4- by 10-foot burning pan at zero wind velocity and at slopes varying from 5 to 50 percent. Because of the small scale of the experiments, ponderosa pine needles were cut to a uniform length of 1½ inches to produce a slow-burning, uniform fuel. It was impractical in these studies to measure perimeter increase, linear spread being used instead. Previous studies had shown that linear spread was closely related to increase

in perimeter, and that generalizations true of one were applicable to the other.

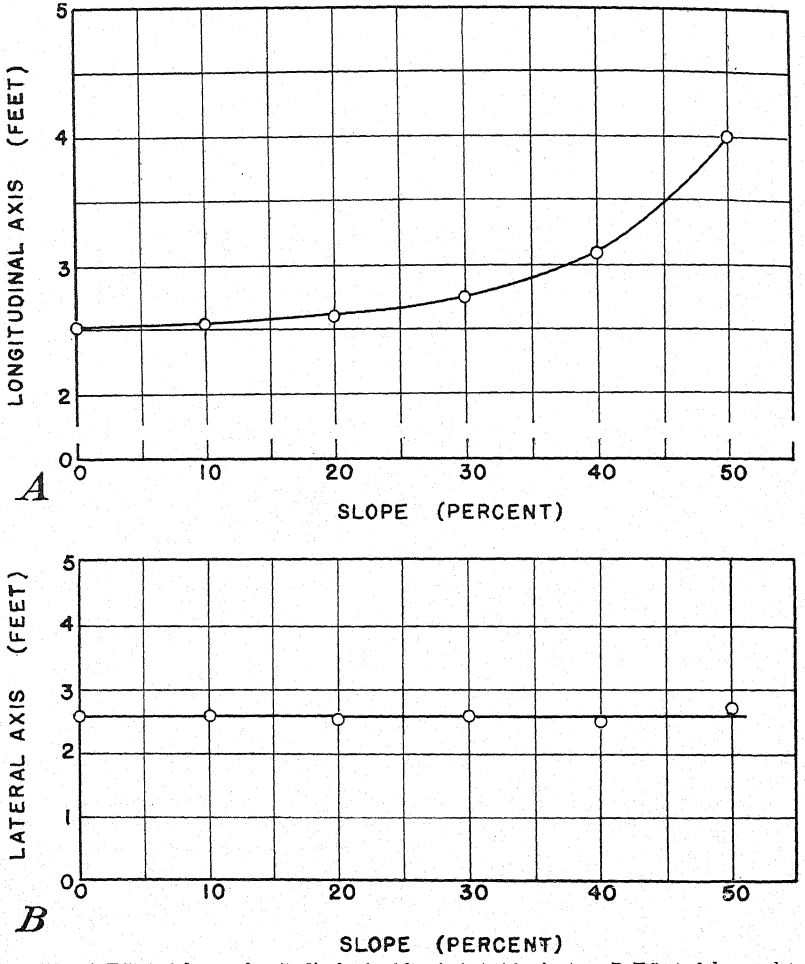


FIGURE 12.—*A*, Effect of slope on longitudinal axis of fires in feet at 3 minutes. *B*, Effect of slope on lateral axis of fires in feet at 3 minutes. Basis for *A* and *B*: 59 laboratory fires under constant moisture content and 0 wind velocity.

TABLE 3.—*Values of k′, k″, K′, and K″, for calculating rate of perimeter increase and total perimeter, equations 3 and 4*

Time (minutes)	k'	k''	K'	K''	Time (minutes)	k'	k''	K'	K''
2	0.35	0.26	0.69	0.52	13	0.41	1.85	4.85	16.44
3	.35	.74	1.04	1.26	14	.41	1.89	5.26	18.33
4	.36	1.00	1.40	2.26	15	.42	1.94	5.68	20.27
5	.36	1.20	1.76	3.46	16	.42	1.98	6.10	22.25
6	.37	1.33	2.13	4.79	17	.43	2.02	6.53	24.27
7	.37	1.44	2.50	6.23	18	.43	2.05	6.96	26.32
8	.38	1.53	2.88	7.76	19	.44	2.08	7.40	28.40
9	.38	1.61	3.26	9.37	20	.44	2.11	7.85	30.51
10	.39	1.68	3.65	11.05	21	.45	2.14	8.30	32.65
11	.40	1.74	4.05	12.79	22	.45	2.16	8.75	34.81
12	.40	1.80	4.45	14.59					

The important contributions of the laboratory study were (1) an indication of the type of curve representing the effect of slope on increase on the longitudinal axis and (2) evidence that slope had apparently no effect on the spread of fire at right angles to the slope direction; these indications are illustrated in figure 12. This study did not result, however, in any quantitative evaluation of the relative effect of slope, moisture, and wind upon rate of spread.

The field study comprised 46 fires at slopes of 10 to 30 percent. These were too few to permit the type of analysis used in determining

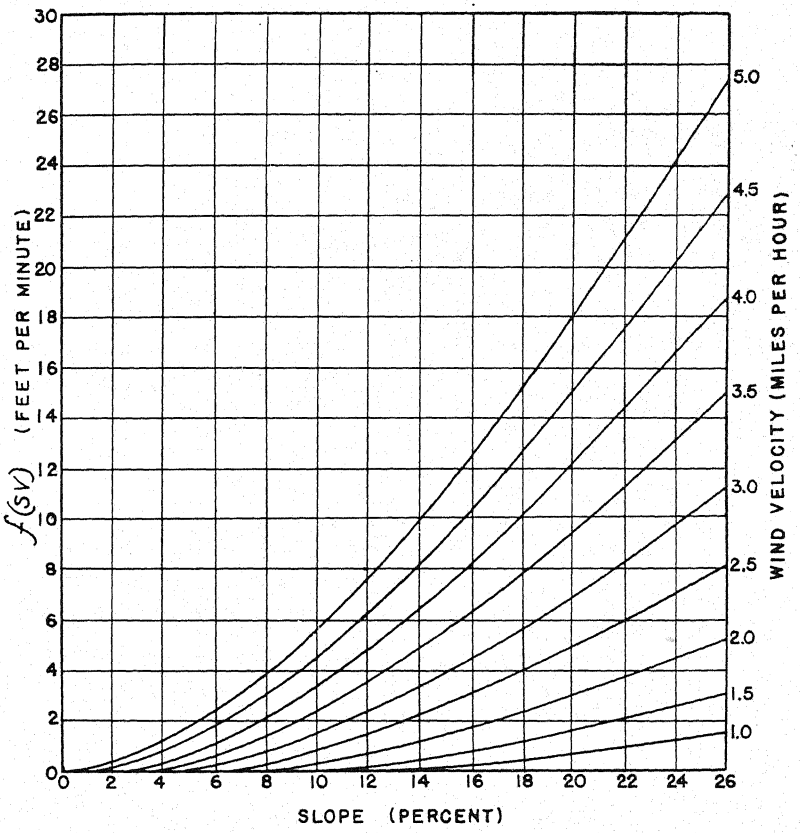


FIGURE 13.—Probable increase in rate of spread for slope fires over values for level ground for various slopes and wind velocities.

wind, moisture, and time relationships. It was decided, therefore, to determine the approximate relation of slope and perimeter through the use of the relationships already determined for fires on level ground, considering the deviations from the level ground perimeters as the influence of slope. When this was attempted, it was found that the size of the deviations depended largely upon the wind velocity at the period, or, in other words, that slope and wind were jointly correlated. Accordingly, the data were classified on the two variables, wind and slope, and deviations for each slope and wind-velocity class determined. The resulting relationships are shown in figure 13.

It seems apparent from a consideration of these curves that the influence of slope on rate of spread is inconsiderable at low wind velocities but that with higher wind, slope becomes increasingly important. The indicated increase due to slope may in many cases approach the original values on level ground. For instance, at moisture content of 5 percent, wind 4 miles per hour, and time 20 minutes, the rate of spread on level ground is 12.6 feet per minute. Under the same conditions but with a slope of 20 percent, the correction to be applied is 12.2 feet, resulting in a total indicated increase of 24.8 feet.

The slope measurements used in the analysis were the slope percent in the direction of the greatest spread, which corresponded closely to the prevailing wind direction. The relationships shown on slope are based on relatively scant data and are therefore not of comparable accuracy to the wind and moisture curves.

As a result of the analysis explained above, equation 2 may now be rewritten

$$p_t=6.35-k'\ M+k''\ V+f(SV) \qquad (3)$$

Values of $f(SV)$ may be obtained from table 4.

ESTIMATING TOTAL PERIMETER

It is apparent that, for any values of wind and moisture within the range of data available, it is possible to calculate the total perimeter at any time interval up to 22 minutes from the relationships already explained and expressed in equation 3.

TABLE 4.—*Values of $f(SV)$ for calculating rate of perimeter increase and total perimeter, equations 3 and 4*

V=wind velocity per hour (miles)	S=slope in percent as indicated						V=wind velocity per hour (miles)	S=slope in percent as indicated					
	0	5	10	15	20	25		0	5	10	15	20	25
0	0	0	0	0	0.1	0.2	3.0	0	0.2	1.6	4.0	7.0	10.5
0.5	0	0	0	0	.2	.5	3.5	0	.4	2.5	5.6	9.5	13.9
1.0	0	0	0	.2	.6	1.2	4.0	0	.8	3.4	7.3	12.2	17.7
1.5	0	0	.2	.7	1.6	2.8	4.5	0	1.3	4.5	9.2	15.0	21.6
2.0	0	0	.4	1.6	3.0	4.8	5.0	0	1.8	5.6	11.2	18.0	25.8
2.5	0	0	.9	2.6	4.8	7.5							

An equation to express total perimeter with constant wind and moisture conditions directly may be derived from equation 3, as follows:

$$P_t=6.35t-(K_t')M+(K_t'')V+f(SV)t$$

in which

P_t=total perimeter at a particular time
t=time in minutes from start of fire
V=wind velocity in miles per hour
K_t'=factor to be multiplied by moisture content (table 3)
M=moisture content in percent
K_t''=factor to be multiplied by wind (table 3)
$f(SV)$=factor to be multiplied by time for slope fires (table 4)

The derivation of the above expression is as follows:

From equation 3, the perimeter at end of 2 minutes would be

$$p_2=2(6.35)-2k_2'M+2k_2''V+2f(SV)$$

and the perimeter increase in the third minute would be

$$p_3=6.35-k_3'M+k_3''V+f(SV)$$

for the fourth minute

$$p_4=6.35-k_4'M+k_4''V+f(SV)$$

and for the nth minute

$$p_t=6.35-k_t'M+k_t''V+f(SV)$$

Total perimeter at end of any time would be

$$P_t=p_2+p_3+p_4+\text{----}+p_t$$

Adding the above equations and factoring

$$P_t=6.35t-(2k_2'+k_3'+k_4'+\text{----}+k_t)M+(2k_2''+k_3''+k_4''+\text{----}+k_t'')V+f(SV)t$$

Let $K_t'=(2k_2'+k_3'+k_4'+\text{----}+k_t')$ and $K_t''=(2k_2''+k_3''+k_4''+\text{----}+k_t'')$

Then, as already expressed,

$$P_t=6.35t-(K_t')M+(K_t'')V+f(SV)t \qquad (4)$$

Values of K_t' and K_t'' have also been arranged in table 3.

Values of $f(SV)$ have been arranged in table 4 for ease in computation.

To illustrate the manner in which total perimeter increases with time, figures 14 to 17 have been prepared. These figures illustrate the characteristic behavior noted by Show (*6*).

ACCURACY OF ESTIMATE

To determine the error of estimate of the derived predictions from the derived relationships, 350 cases of rate of spread for a 2-minute period were drawn at random from the 1,153 cards for fires on level ground and estimates from the appropriate equation compared to the observed values. The results follow.

Fires on level:		
Average perimeter increases	feet	11. 4
Standard deviation of average perimeter increase	do	6. 2
Standard error of estimate	do	4. 3
Index of alienation		0. 69
Index of correlation		0. 72

As there were in all only 372 2-minute cases of fires on slopes, the entire sample was used in the computation of the measures listed below:

Fires on slopes:		
Average perimeter increases	feet	22. 9
Standard deviation of average	do	16. 5
Standard error of estimate	do	12. 7
Alienation index		0. 77
Correlation index		0. 64

The absence of higher correlation is due in part to inadequacy of measurement of perimeter, moisture, and wind, as well as to failure to include all the factors affecting the spread of fire. On a fast-burning fire it is impossible to approximate the location of the line closer than 1 foot, and this error is magnified in terms of perimeter. Measurements of moisture are only average values for the entire plot

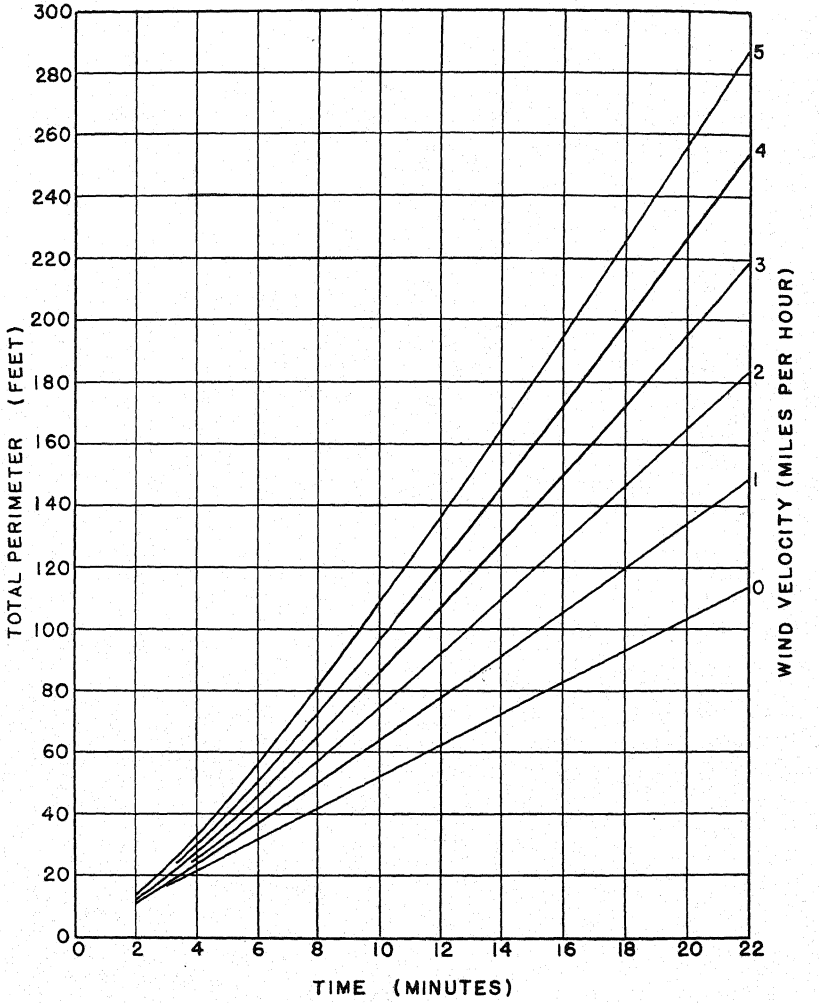


FIGURE 14.—Probable total perimeter of fires burning under various constant wind velocities at moisture content 3 percent.

and cannot be expected to approximate closely the moisture content of the particular portion of the litter in which the fire is spreading at a given time interval. Measurements of wind velocity, although taken by 2-minute time intervals, may also fail to show accurately the wind movement near the head of the fire. These errors are not cumulative but compensating, and greater correlation may accordingly be expected when the relationships are used in the estimation of total per-

imeter. That improved errors of estimate result when total perimeter is used instead of perimeter increase, is shown by table 5, in which indexes of correlation and errors of estimate have been derived for each 2-minute period from a comparison of the estimated and actual total perimeters. Table 5 also gives comparisons of actual and estimated perimeters on slope.

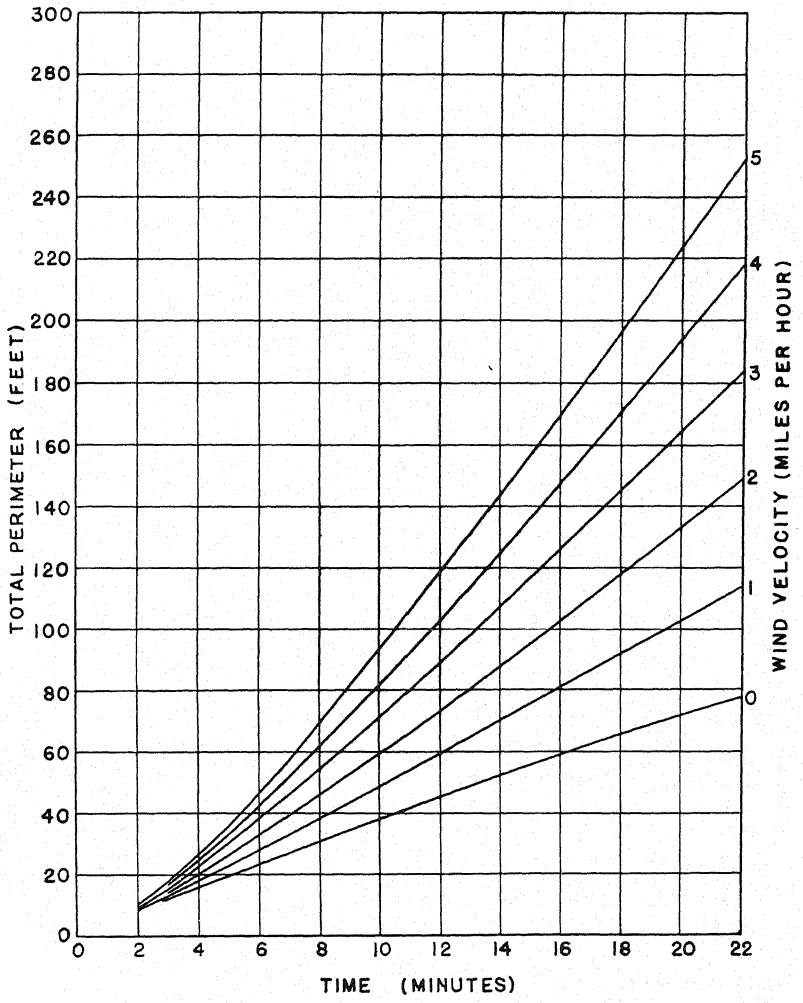


FIGURE 15.—Probable total perimeter of fires burning under various constant wind velocities at moisture content 7 percent.

The measurements indicate that, although it is not possible to estimate rate of perimeter increase with a high degree of accuracy, yet, when the relationships derived are applied to estimates of total perimeter, a smaller error in proportion to the observed or dependent value results. This fact has considerable bearing on the practical side of the problem.

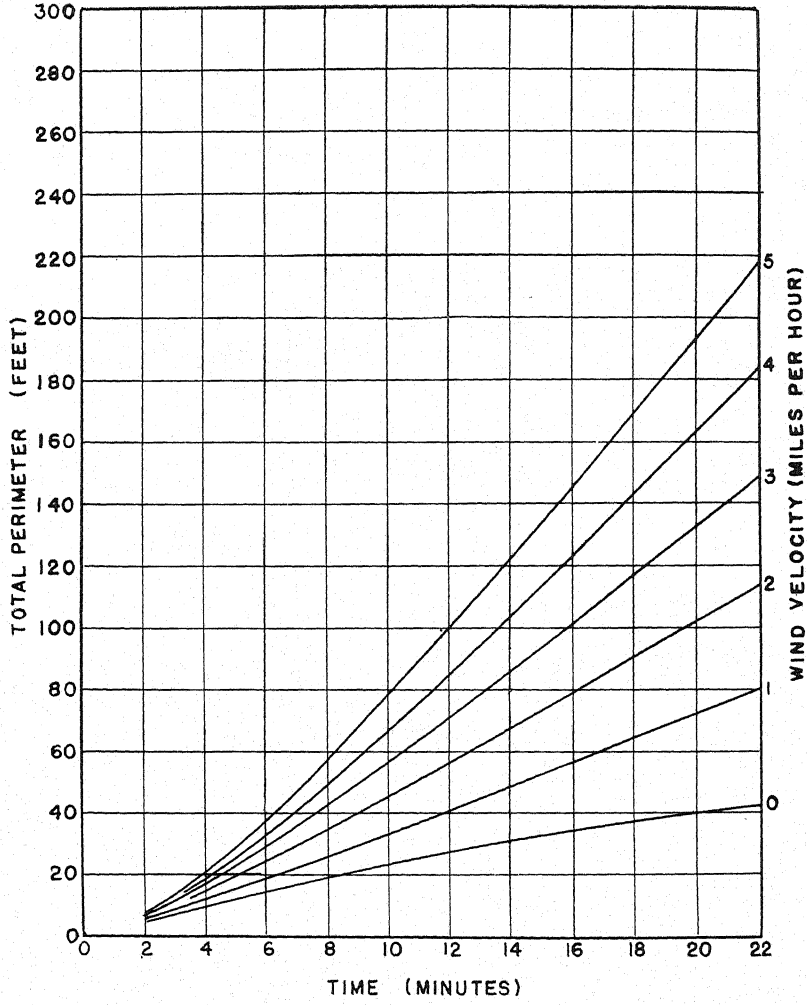


FIGURE 16.—Probable total perimeter of fires burning under various constant wind velocities at moisture content 11 percent.

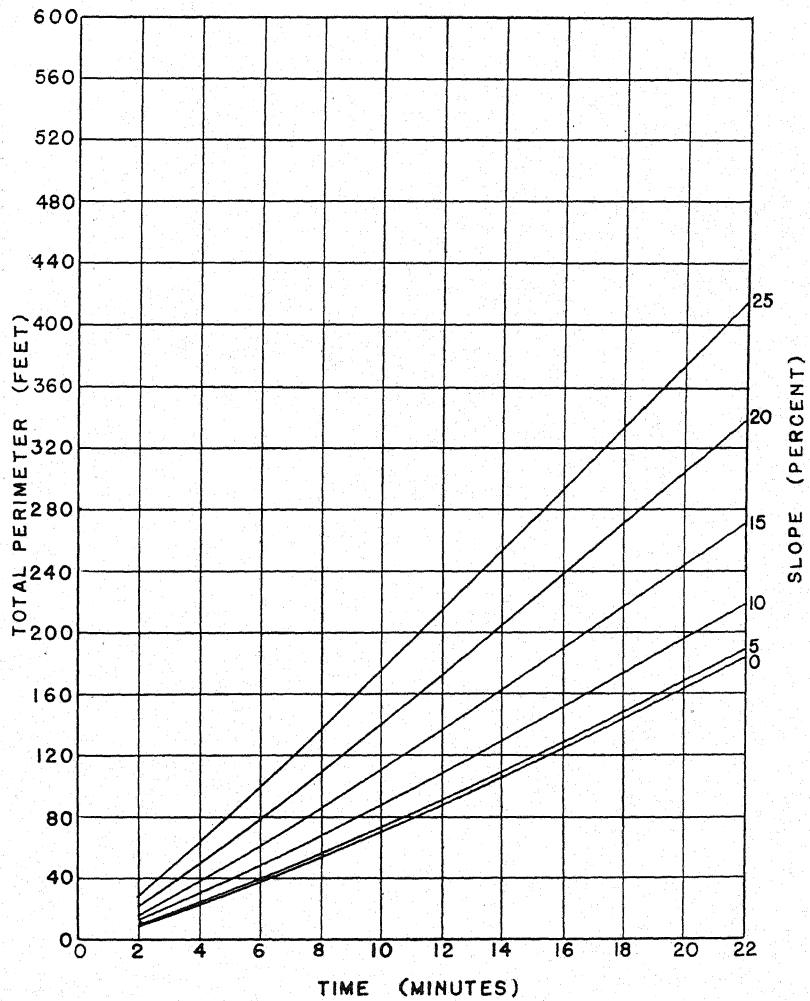


FIGURE 17.—Probable total perimeter of fires on various slopes for moisture content 7 percent and wind velocity 3 miles per hour.

TABLE 5.—*Summary of results in the application of the derived relationships to the computation of total perimeter of test fires on level and sloping ground at each 2-minute time interval from start of fire*

Time (minutes)	Cases	Average perimeter		Standard deviation of average actual perimeter	Standard error of estimate		Alienation index	Correlation index
		Actual	Calculated					
	Number	*Feet*	*Feet*	*Feet*	*Feet*	*Percent*		
FIRES ON LEVEL GROUND								
2	118	7.5	7.6	2.6	2.1	28.0	0.81	0.59
4	118	17.7	17.7	6.4	4.5	25.4	.70	.71
6	118	28.9	28.6	9.8	6.2	21.5	.63	.78
8	118	40.0	40.0	15.0	9.4	23.5	.63	.78
10	118	51.2	51.6	18.6	11.1	21.7	.60	.80
12	117	63.5	63.0	22.6	13.7	21.6	.61	.79
14	114	75.4	74.7	27.1	16.0	21.2	.59	.81
16	109	87.0	86.1	31.5	16.5	19.0	.52	.85
18	96	97.7	96.5	38.9	19.2	19.7	.49	.87
20	71	102.3	102.8	40.9	17.2	16.8	.42	.91
22	56	112.6	112.6	51.2	19.7	17.5	.38	.92
FIRES ON SLOPING GROUND								
2	46	12.1	15.5	7.2	6.8	56.2	0.94	0.34
4	46	32.4	34.9	18.5	10.2	31.5	.55	.84
6	46	58.5	57.4	33.2	18.4	31.5	.55	.84
8	46	87.2	82.8	52.1	30.4	34.9	.58	.81
10	42	105.5	102.4	64.5	38.5	36.5	.60	.80
12	36	116.2	122.2	50.6	35.5	30.6	.71	.70
14	31	128.1	139.8	52.5	37.8	29.5	.72	.69
16	27	144.3	166.3	59.5	41.6	28.8	.70	.71

SUMMARY

In this study of rate of spread of small surface fires in selected even-aged second-growth stands of *Pinus ponderosa*, records were analyzed of about 160 fires with burning periods of 16 to 22 minutes.

The influence of both moisture and wind is described as linear, with the slope of the curves varying with the time factor. Where fires burned with low wind, the maximum rate of spread as controlled by the moisture content was soon attained. With higher velocities, the period required to attain maximum rate of spread increased, and in some cases did not yet reach that maximum at the end of the 22-minute period, the time limit of the test fires.

The effect of slope on rate of spread was found to be curvilinear and largely dependent for its influence upon the occurrence of winds in the direction of the slope.

Empirical formulas derived from the smoothed curves permit estimates of rate of perimeter increase or total perimeter at any time interval within the limits of the data. The generalizations made appear highly significant, and prediction of total perimeter may be possible within a reasonable error of estimate.

Extension of rate-of-spread studies to other types is dependent to a large extent upon research leading to methods of evaluation of the factor of fuel variation. With progress in present studies of fuels, similar studies in more complex forest types are contemplated.

LITERATURE CITED

(1) Buck, C. C.
1938. THE SOLVENT DISTILLATION METHOD FOR DETERMINING THE MOISTURE CONTENT OF FOREST FUELS. Jour. Forestry. Unpublished manuscript.
(2) Ezekiel, M.
1930. METHODS OF CORRELATION ANALYSIS. 427 pp., illus. New York and London.
(3) Gisborne, H. T.
1928. MEASURING FOREST FIRE DANGER IN NORTHERN IDAHO. U. S. Dept. Agr. Misc. Pub. 29, 64 pp., illus.
(4) Hawley, L. F.
1926. THEORETICAL CONSIDERATIONS REGARDING FACTORS WHICH INFLUENCE FOREST FIRES. Jour. Forestry 24: 756–763.
(5) Mills, F. C.
1925. STATISTICAL METHODS APPLIED TO ECONOMICS AND BUSINESS. 604 pp., illus. New York.
(6) Show, S. B.
1919. CLIMATE AND FOREST FIRES IN NORTHERN CALIFORNIA. Jour. Forestry 17: 965–979, illus.
(7) ——— and Kotok, E. I.
1925. WEATHER CONDITIONS AND FOREST FIRES IN CALIFORNIA. U. S. Dept. Agr. Cir. 354, 24 pp., illus.
(8) ——— and Kotok, E. I.
1930. THE DETERMINATION OF HOUR CONTROL FOR ADEQUATE FIRE PROTECTION IN THE MAJOR COVER TYPES OF THE CALIFORNIA PINE REGION. U. S. Dept. Agr. Tech. Bull. 209, 47 pp., illus.
(9) Stickel, P. W.
1931. THE MEASUREMENT AND INTERPRETATION OF FOREST FIRE-WEATHER IN [THE] WESTERN ADIRONDACKS. N. Y. State Col. Forestry, Syracuse Univ., Tech. Pub. 34, 115 pp., illus.

ANATOMY OF THE LEAF AND STEM OF GOSSYPIUM [1]

By IRMA E. WEBBER [2]

Collaborator, Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

Because of the great commercial importance of cotton fibers, their structure at various stages of development has been carefully studied. An indication of the work that has been done along this line may be gained from the papers of Dischendorfer (*6*),[3] Youngman and Pande (*53*), and Kerr (*21*), which give a number of literature citations. Structure of the seed (*14, v. 3; 32; 33; 34; 35; 36; 37*) and that of the boll (*14, v. 3; 40*) are intimately connected with fiber production and have received considerable attention. The anatomy of the cotton flower has also been investigated to some extent (*7; 10; 14, v. 3–4*).

The structure of the vegetative organs of *Gossypium*, although closely connected with the various plant functions and hence influencing fiber yield, has received comparatively little attention. Some anatomical characteristics of seedlings of *G. hirsutum* × *G. barbadense* were depicted by Heim de Balsac (*14, v. 1*). The relation of the various reproductive and vegetative parts of mature cotton plants of different types has been repeatedly described (*4, 5, 16, 38*), and an attempt has been made by Zaitzev (*54*) to ascertain the gross morphological characteristics of Old World cottons with a haploid chromosome number of 13 and New World cottons with a haploid chromosome number of 26, but descriptions of the histology of the vegetative organs are wholly inadequate. A brief account of the anatomy of the various vegetative organs of an unnamed species of *Gossypium* was given by Flatters (*9*), rather detailed reports of the microscopic structure of the roots (*11*) and the transition region (*44*) of *G. hirsutum* L. have been made, the ontogeny of the main stem and fruiting branches has been traced (*10*), and some histological characteristics of leaves and stems of *G. herbaceum* L., *G. barbadense* L. (*14, v. 3*), and *G. hirsutum* × *barbadense* (*14, v. 2*) have been figured; but there is no consecutive account of the anatomical characteristics of the vegetative organs of the main groups of species or of the genus as a whole. Since there is considerable disagreement concerning the taxonomy of the genus and since microscopic and endomorphic characters when considered in conjunction with macroscopic exomorphic ones are often helpful in solving taxonomic problems, the present anatomical study of the genus *Gossypium* was undertaken.

MATERIAL AND METHODS

Although this investigation was limited to nonfruiting branches, special attention was paid to anatomical characteristics that might be useful in distinguishing between American wild cottons with 13 haploid chromosomes (*12, 19, 20*) which have been excluded from the genus

[1] Received for publication October 13, 1937; issued August 1938.

[2] The material examined was made available for study through the courtesy of T. H. Kearney and J. M. Webber, of this Division.

[3] Italic numbers in parentheses refer to Literature Cited, p. 283.

Gossypium by some taxonomists, Old World cottons with 13 haploid chromosomes, and New World cottons with 26 haploid chromosomes. For comparative purposes a limited number of *Gossypium* hybrids and representatives of other genera of the Hibisceae were also included in the study. The species examined and the groups to which they belong are:

Old World cottons with 13 haploid chromosomes: *Gossypium africanum* Watt, *G. anomalum* Wawra and Peyr., *G. arboreum* L., *G. cernuum* Tod., *G. herbaceum* L., *G. intermedium* Tod., *G. nanking* Meyen, *G. neglectum* Tod., *G. sanguineum* Hasskl., *G. stocksii* Mast., *G. sturtii* F. Muell., *G. transvaalense* Watt.

American wild cottons with 13 haploid chromosomes: *Gossypium armourianum* Kearney, *G. davidsonii* Kellogg, *G. harknessii* Brandeg., *G. klotzschianum* Anderss., *G. thurberi* Tod. (*Thurberia thespesioides* A. Gray).

New World cottons with 26 haploid chromosomes: *Gossypium barbadense* L., *G. brasiliense* Macf., *G. contextum* Cook and Hubbard, *G. darwinii* Watt, *G. hirsutum* L., *G. hopi* Lewton, *G. peruvianum* Cav., *G. purpurascens* Poir., *G. schottii* Watt, *G. tomentosum* Nutt.

Doubtful species of *Gossypium* and members of related genera: *Gossypium kirkii* Mast., *Alyogyne hakeaefolia* (Giord.) Alef., *Erioxylum aridum* Rose and Standl., *Hibiscus brackenridgei* A. Gray, *H. tiliaceus* L., *Kokia drynarioides* (Seem.) Lewton, *K. rockii* Lewton, *Lagunaria patersonii* G. Don, *Thespesia lampas* (Cav.) Dalz., *T. populnea* Soland., and *Shantzia garckeana* Lewton.

All of the material examined was taken from plants grown in the field at the Rubidoux Laboratory,[4] Riverside, Calif. Much of the material was sectioned when fresh, but some of it was first killed and fixed in formalin-acetic alcohol and embedded in paraffin. Safranine was used to stain some sections; the remainder were left unstained.

LEAF ANATOMY

EPIDERMIS

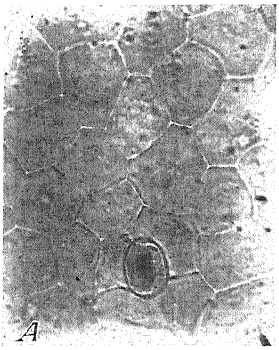
The ordinary epidermal cells over chlorenchyma may be straight-walled or very sinuate in surface view. In most species their lateral walls are much less sinuous in the upper epidermis than in the lower epidermis, where stomata are more numerous (pl. 1, *A*, *B*). However, in some species the lateral walls of epidermal cells are characteristically rather straight on both upper and lower leaf surfaces (*C*, *D*). On vertically transcurrent veins the epidermal cells are longer and narrower than elsewhere on the lamina, the long axis of the cell being parallel to the course of the vein (*E*). Over lysigenous cavities in the mesophyll, the epidermal cells are concentrically arranged and generally smaller than the surrounding cells (*F*).

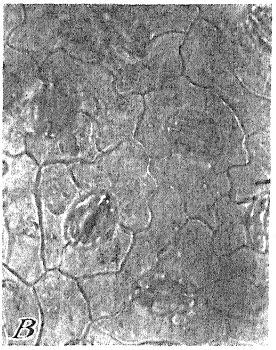
According to Flatters (*9*, *p. 43*) "The epidermis of the upper [leaf] surface consists of closely-packed cells with resin-cells distributed among them * * *." What he here designates as "resin-cells" is not clear to the writer. In his figure of a cross section of a stem the lysigenous cavities are so labeled, but their absence in the epidermis

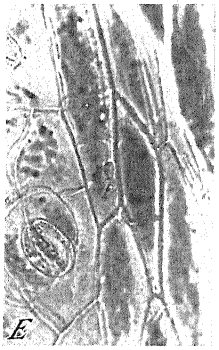
[4] Maintained cooperatively by the U. S. Department of Agriculture and the California Agricultural Experiment Station.

EXPLANATORY LEGEND FOR PLATE 1.

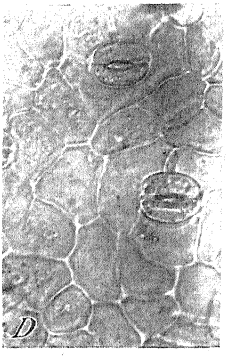
Epidermis of *Gossypium* leaves. × 312. *A–J*, Surface views showing comparative shape and size of ordinary epidermal cells and stomata. *A* and *B*, *G. brasiliense: A*, From upper side of leaf; *B*, from lower side of leaf. *C* and *D*, *G. harknessii: C*, From upper side of leaf; *D*, from lower side of leaf. *E* and *F*, *G. peruvianum: E*, Epidermis of a vein; *F*, epidermis over a lysigenous cavity in the mesophyll. *G–J*, Epidermis from lower surfaces of leaves: *G*, *G. hirsutum* var. Acala; *H*, *G. hirsutum* × *G. cernuum*; *I*, *G. armourianum*; *J*, *G. hirsutum* × *G. armourianum*. *K–M*, Leaf sections showing position of stomata: *K*, *G. contextum*, lower epidermis; *L*, *G. arboreum*, upper epidermis; *M*, *G. harknessii*, upper epidermis.

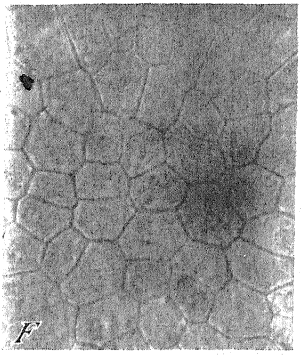




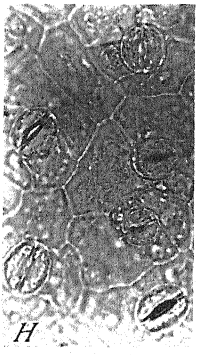


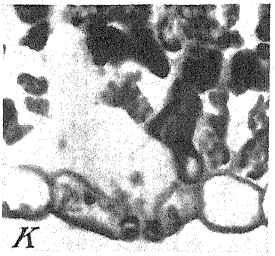




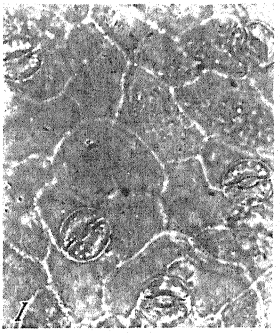




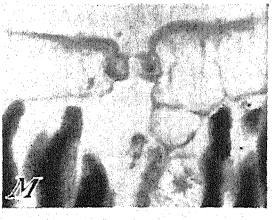




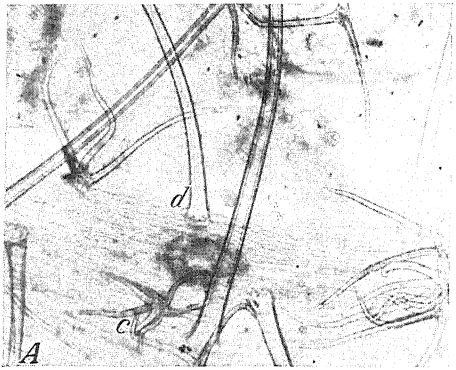


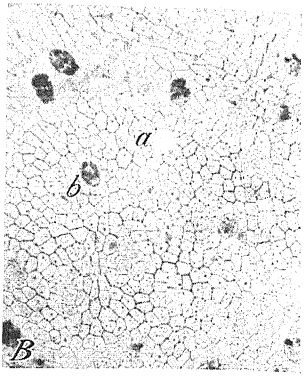


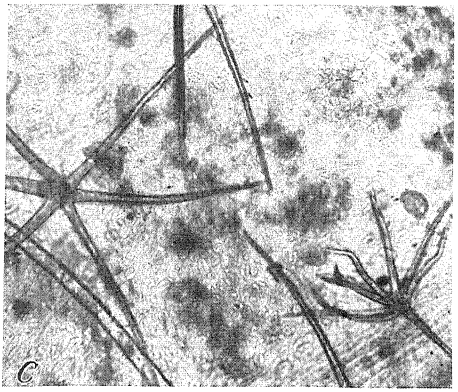


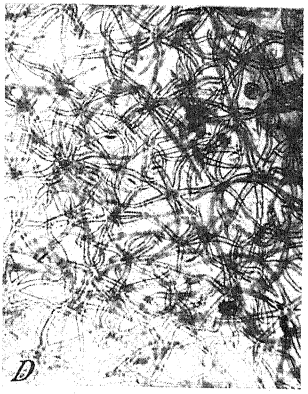


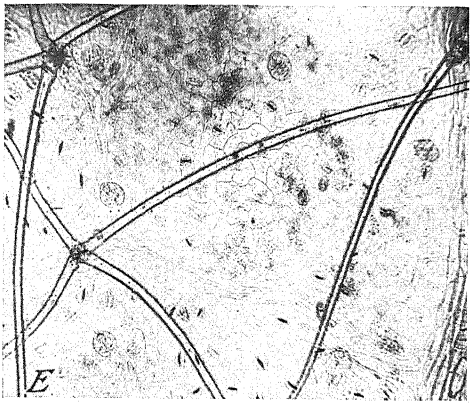
FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

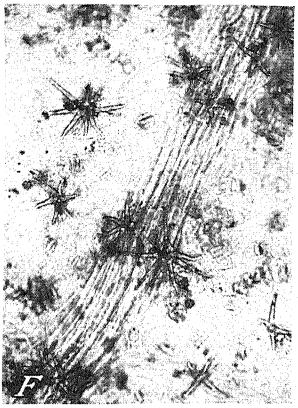


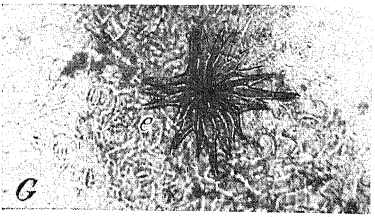


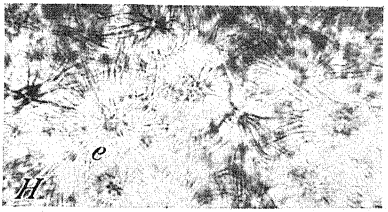












FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

of leaves suggests that he either did not distinguish clearly between epidermal and subepidermal tissue, or applied the term "resin-cells" to some other structure as well as to the lysigenous cavities. In the latter case, ordinary epidermal cells with colored contents, mucilage cells, or minute, depressed capitate hairs may be meant.

Ordinary epidermal cells with colored, usually purplish or reddish, cell sap are of scattered occurrence in most species of *Gossypium*. In green-leaved plants they commonly adjoin the guard cells of stomata and are not nearly so numerous as the hyaline epidermal cells. In reddish-leaved plants, such as *G. purpurascens* and the Red Acala variety of *G. hirsutum*, they are not restricted to the vicinity of stomata and may be more numerous than hyaline epidermal cells. The inheritance of red plant color in cotton plants has been discussed by Ware (*49*).

Although mucilage cells were reported by Dumont (*8*) as completely wanting in *Gossypium* and as very rare throughout the Hibisceae, according to Kuntze (*22*) they are characteristic of both the upper and the lower epidermis of the leaves of all Hibisceae. They are apparently lacking or inconspicuous in the epidermis of *Kokia*, *Shantzia*, *Erioxylum*, and most species of *Gossypium*, but fairly numerous in that of *G. kirkii* (pls. 2, *B*, *a*; 3, *M*, *a*), *G. klotzschianum* (pl. 3, *B*, *a*), *Thespesia*, *Hibiscus*, and *Lagunaria*. As a rule, they are considerably larger than the adjoining cells, and more common in the upper than in the lower epidermis. The distribution and structure of mucilage cells in a number of malvaceous genera, chiefly representative of tribes other than the Hibisceae, have been discussed by Trécul (*46*), Walliczek (*48*), and Nestler (*28*).

Heim de Balsac (*14*, *v. 3*) depicted stomata in the lower but not the upper epidermis of the leaves of *Gossypium herbaceum* and *G. barbadense*, while stomata were reported by Flatters (*9*) as few or absent in the upper epidermis of the leaves of *Gossypium* and by Kuntze (*22*) as lacking in the upper epidermis of *G. drynarioides* Seem. The latter species is now referred to the genus *Kokia* and was found by the writer to have a few widely scattered stomata on the upper leaf surface. In all species of *Gossypium* examined, stomata are present on both upper and lower leaf surfaces but are more numerous on the lower surface. In some species the difference between the average number of stomata per square millimeter of the upper and the lower epidermis of a leaf is very marked, while in others it is comparatively slight. For example, when stomatal counts were made near the centers of mature leaf blades in areas free from large veins, the average number of stomata per square millimeter of upper and lower leaf surfaces were, respectively, 122 and 159 in *G. harknessii*, and 40 and 218 in *G. peruvianum*. The correlation indicated by Kuntze for most Malvaceae between a dense coating of hairs and the presence of stomata in the upper epidermis, or dense hairy covering and relatively high stomatal number in the lower epidermis, does not hold true in *Gossypium*. In leaves of the species examined by the writer the average number of stomata per square millimeter of the upper epidermis ranged

EXPLANATORY LEGEND FOR PLATE 2.

Leaf epidermis of *Gossypium* and related genera showing various types of hairs. × 80. *A*, *G. intermedium*; *B*, *G. kirkii*; *C*, *G. sanguineum*; *D*, *G. tomentosum*; *E*, *G. peruvianum*; *F*, *G. thurberi*; *G*, *Thespesia populnea*; *H*, *Lagunaria patersonii*. *a*, Mucilage cell; *b*, capitate hair; *c*, stellate hair; *d*, simple hair; *e*, peltate scale.

89150—38——3

from 40 in *G. peruvianum* to 170 in *G. intermedium*, while average stomatal numbers per square millimeter of the lower epidermis varied from 80 in *G. tomentosum* to 280 in *G. anomalum*.

Within the genus *Gossypium* the stomatal apparatus of leaves varies from roundish (pl. 1, *H*, *I*) to elliptic (*A*, *B*, *C*, *D*, *E*, *G*, *J*) in surface view, the latter form dominating. Twin and malformed stomata are occasionally present, being somewhat more numerous in certain hybrids than in their parents. The normal stomatal apparatus of mature leaves varies in average length from about 24μ to 32μ and in average width from about 16μ to 24μ. Within a species there are no appreciable differences in size or shape between stomata of the upper leaf surface and those of the lower leaf surface, some variation generally occurring on both surfaces. Because of the similarity of stomata in most of the species, stomatal size in interspecies hybrids of *Gossypium* is generally of little significance. At times, however, when a species with relatively large stomata (*G*) and one with small stomata (*I*) are crossed, stomatal size of the hybrid is clearly intermediate between that of the parents (*J*). Some such hybrids show a wider range in stomatal size than most of the species (*H*). Stomata may be level with the ordinary epidermal cells (*L*), slightly above the general epidermal surface (*K*), or slightly below it (*M*), those on a single leaf often being in more than one position.

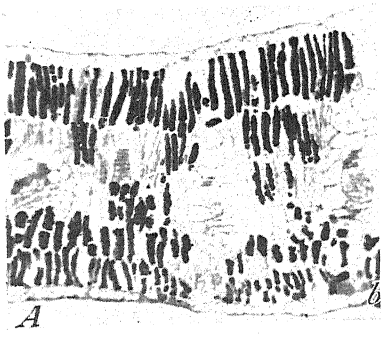
Exclusive of epidermal outgrowths, the thickness of the epidermis on mature leaves of *Gossypium* varies considerably. In some species and hybrids, notably *G. armourianum* (pl. 3, *A*), *G harknessii*, *G. thurberi* (*C*), G. *armourianum* × *G. harknessii*, *G. thurberi* × *G. sturtii*, *G. sturtii* × *G. harknessii*, and *G. hirsutum* × *G. sturtii*, the upper and lower epidermis of a leaf are of approximately the same thickness. In such plants the average thickness of the leaf epidermis over chlorenchyma varies from about 16μ to 32μ. In the majority of species the upper epidermis of a leaf is thicker than the lower epidermis. The difference in thickness may be relatively slight as in *G. barbadense*, *G. peruvianum* (*E*), and *G. anomalum* (*I*), or considerable as in *G. klotzschianum* (*B*), *G. hirsutum* (*F*), and *G. herbaceum* (*J*). In leaves with upper and lower epidermis of different thicknesses, the upper epidermis varies in average thickness from about 14μ to 32μ, and the thickness of the lower epidermis ranges from about 10μ to 32μ. Over large veins the epidermal cells often have thicker outer walls than elsewhere on the lamina.

Three types of epidermal outgrowths occur on the leaves and stems of *Gossypium*. The ontogeny of each hair type has been traced by Youngman and Pande (*53*) and shows a marked similarity to that of comparable trichomes described by Rauter (*30*) in *Malva*.

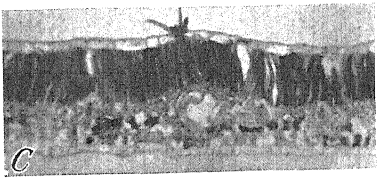
Multicellular capitate hairs, sometimes called glandular hairs (*43*), club-shaped bodies (*53*), or septate papillae (*45*), were observed on both the upper and the lower leaf surfaces of all species of *Gossypium* examined. They are more numerous than other types of trichomes on the mature leaves of *G. armourianum*, *G. harknessii*, *G. barbadense*, *G. brasiliense*, *G. contextum*, *G. darwinii*, *G. hirsutum*, *G. hopi*, *G.*

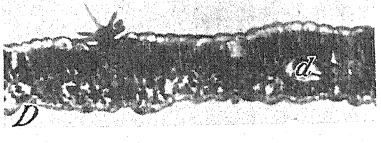
EXPLANATORY LEGEND FOR PLATE 3.

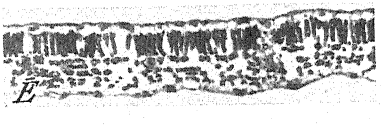
Cross sections of leaf blades showing structure of the epidermis and mesophyll. ×80. *A*, *Gossypium armourianum*; *B*, *G. klotzschianum*; *C*, *G. thurberi*; *D*, *Eriorylum aridum*; *E*, *G. peruvianum*; *F*, *G. hirsutum*; *G*, *G. tomentosum*; *H*, *G. barbadense*; *I*, *G. anomalum*; *J*, *G. herbaceum*; *K*, *G. cernuum*; *L*, *G. sturtii*; *M*, *G. kirkii*. *a*, Mucilage cell; *b*, capitate hair; *c*, stellate hair; *d*, lysigenous cavity in mesophyll.



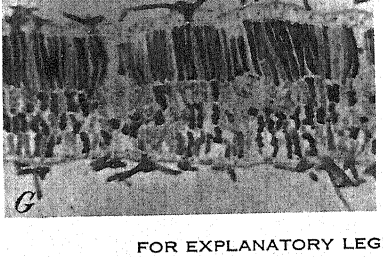


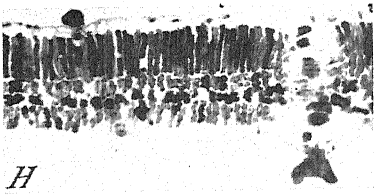


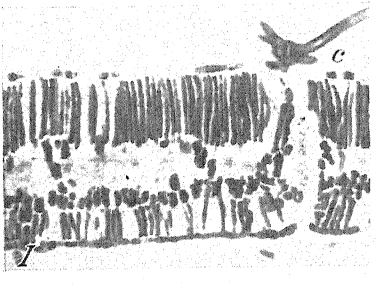




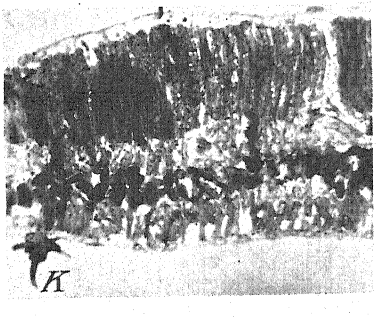


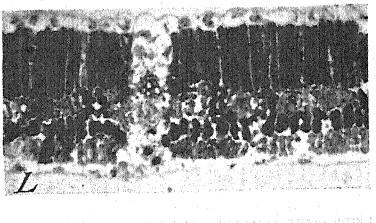


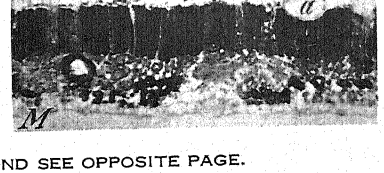




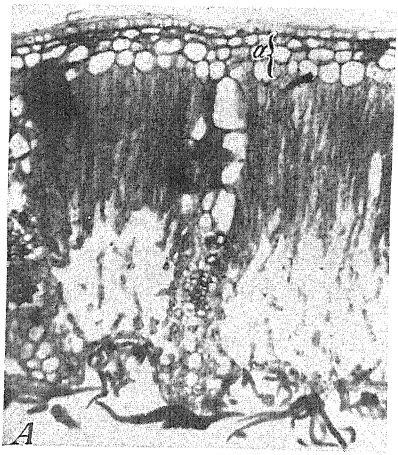




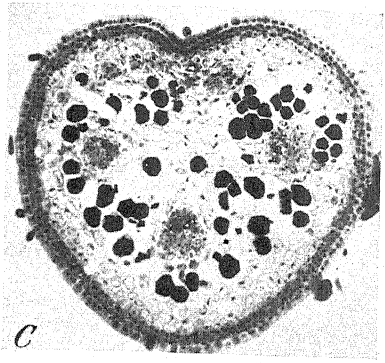


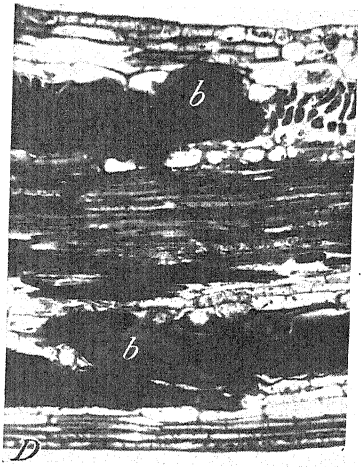


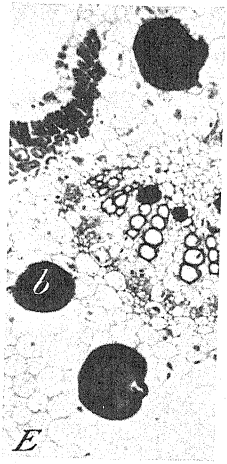
FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

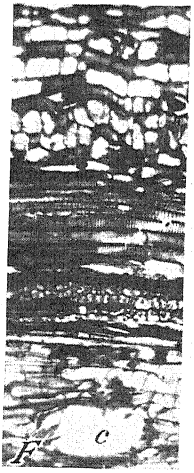


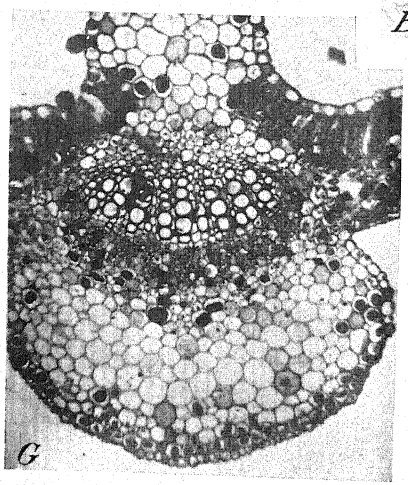


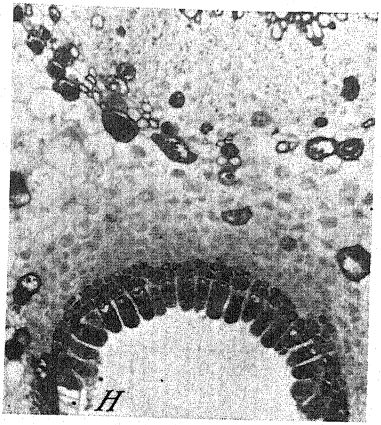












FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

peruvianum, *G. purpurascens*, *G. schottii*, and *G. kirkii* (pl. 2, *B*), but are comparatively rare even in young leaves of *G. sturtii*. They vary considerably in size, shape, color of contents, and the extent to which their bases are depressed below the level of non-trichome-bearing cells (pl. 3, *A*, *B*, *F*, *H*, *I*). They are not confined to the genus *Gossypium*, having been observed also in the genera *Alyogyne* (pl. 4, *C*), *Lagunaria*, *Hibiscus*, *Kokia*, *Thespesia*, and *Shantzia*. Other types of capitate and glandular hairs characteristic of various malvaceous genera have been figured by Janda (*18*).

Stellate hairs, characteristic of the family Malvaceae, were present on the leaves of all species of *Gossypium* examined. Their absence in some species of this genus has been pointed out by Hubbard (*16*), who reported a South American species with simple hairs, and by Youngman and Pande (*53*), who mentioned mutant forms of both Old World and New World cottons without stellate hairs or their derivatives. The stellate hairs of *Gossypium* leaves are commonly more abundant on the lower than on the upper surface, and frequently more abundant along the major veins than elsewhere on the lamina. They are often more conspicuous on the petiole than on the leaf blade. Some indication of their diversity in size, form, and distribution on lower leaf surfaces of various species of *Gossypium* and related genera is given in plates 2, *A*, *C*, *D*, *E*, *F*; 3, *C*, *D*, *G*, *H*, *I*, *J*, *K*; and 4 *A*, *C*.

Simple hairs, apparently derivatives of stellate hairs, are generally scattered among the stellate hairs on the mature leaves of most species of *Gossypium*. Usually they resemble a single ray of a stellate hair in both form and size. However, in some species, for example, *G. africanum*, *G. arboreum*, *G. nanking*, and *G. neglectum*, they are both broader and longer than the individual rays of the stellate hairs with which they are associated (pl. 2, *A*). In species with stellate hairs of two distinct sizes, e. g., *G. sanguineum* (*C*), some of the simple hairs resemble the rays of the large stellate hairs and some resemble those of the small stellate hairs.

No peltate scales, such as are characteristic of the lower surfaces of the leaves of the related species *Thespesia populnea* (pl. 2, *G*) and *Lagunaria patersonii* (*H*), were observed in *Gossypium*. The distribution and characteristics of peltate scales in a number of families have been discussed by Bachmann (*2*).

HYPODERMIS

Of the species listed by Solereder (*43*) under the Malvaceae as having a many-layered epidermis or hypodermis, all but *Gossypium drynarioides* are members of the Bombacaceae. This species, now referred to the genus *Kokia*, resembles *Kokia rockii* in that it does not develop a hypodermis under Riverside conditions (pl. 4, *B*). Although scattered epidermal cells divided by horizontal walls are sometimes present in *Gossypium* and related genera, true hypodermis is apparently lacking in *Gossypium*. However, a well-developed hypodermis occurs in *Lagunaria patersonii* (*A*), which Dumont (*8*) reported,

EXPLANATORY LEGEND FOR PLATE 4.

Leaf sections of *Gossypium* and related genera. × 80. *A–C*, Cross sections showing characteristics of epidermis and mesophyll: *A*, *Lagunaria patersonii*; *B*, *Kokia drynarioides*; *C*, *Alyogyne hakeaefolia*. *D*, *Hibiscus tiliaceus*, longitudinal section of midrib. *E*, *H. brackenridgei*, cross section of midrib. *F*, *G. sanguineum*, longitudinal section of midrib. *G* and *H*, *G. schottii*, cross sections of midribs: *G*, Taken at about midpoint of vein; *H*, taken through nectary. *a*, Hypodermis; *b*, mucilage canal; *c*, lysigenous cavity.

under the name *Lagunea squamea*, as having much elongated epidermal cells often divided transversely.

MESOPHYLL

The predominance of bifacial leaf structure in the Malvaceae has been pointed out by Solereder (*43*). Aside from the bombacaceous species he includes, he reports centric leaf structure only in *Malva parviflora*. In describing the anatomy of cotton leaves, Flatters (*9, p. 43*) states that the upper part of the mesophyll is composed of two layers of palisade cells. "The lower half of the mesophyll is made up of loosely arranged irregular cells with large air spaces between them * * *." The inapplicability of Flatters' description to the mesophyll of all species of *Gossypium* and the commoner occurrence of centric leaf structure than was indicated by Solereder (*43*) are evident from the account of structural differences in mesophyll within the genus *Gossypium* given by Magitt and Magitt (*25*). They stated that palisade parenchyma underlies the epidermis of only the upper surface of the leaf in American cottons and in hybrids between American and Asiatic cottons, but occurs beneath the epidermis on both upper and lower leaf surfaces in Asiatic cottons. This distinction between Old World and New World cottons does not apply to plants grown in the field at Riverside. Specimens examined by the writer showed that leaves of some species of the Old World group (pl. 3, *I, K, L*), of the American group with 26 pairs of chromosomes (*G, H*), and of the wild American group with 13 pairs of chromosomes (*A*) develop palisade underlying the epidermis of both the upper and the lower surface. Within each of these three groups of *Gossypium*, the leaves of some species are characterized by mesophyll composed of palisade tissue underlying the upper epidermis and spongy parenchyma underlying the lower epidermis (*B, C, E, F, J*). A less common type of centric leaf structure than that occurring in *Gossypium* was observed in the cotton relative *Alyogyne hakeaefolia* (pl. 4, *C*).

In structure as well as in distribution, the palisade tissue of *Gossypium* leaves shows greater diversity than was indicated by Flatters (*9*). In the leaves of *G. armourianum* (pl. 3, *A*) and *G. harknessii* there are areas in which palisade tissue extends from the upper to the lower epidermis and is commonly four or five cells thick. The palisade layers are more commonly one cell than two cells thick in leaves with palisade tissue restricted to the upper side or with clearly distinct upper and lower palisade layers. Palisade cells of plants grown in the field vary in length from about 30μ to 220μ and are commonly longer in the upper layer of palisade parenchyma than in the lower layer, when the latter is present.

The spongy parenchyma of *Gossypium* leaves is often more compact than Flatters (*9*) indicated. At times the form and arrangement of spongy parenchyma cells so closely approximates that of weak palisade tissue that a distinction between the two types of tissue is purely arbitrary.

Oblate or spherical cavities, usually 50μ to 170μ in diameter and commonly with dark-purplish or brownish contents (pl. 3, *C, M*), occur in the mesophyll of all species of *Gossypium* examined and in the mesophyll of *Erioxylum aridum* (*D*), *Kokia drynarioides* (pl. 4, *B*), *K. rockii*, *Thespesia lampas*, *T. populnea*, and *Shantzia garckeana*

They were reported by Dumont (*8*) to be of schizogenous origin, but according to the earlier report of Von Höhnel (*15*) on similar cavities in the cotyledons of *Gossypium herbaceum* and the more recent work of Stanford and Viehoever (*45*) on various organs of *G. hirsutum*, they are formed lysigenously. Similar cavities have been referred to under a variety of names, including mucilage glands (*14, v. 2*), mucilage pockets (*8, 22*), resin cells (*9*), resin glands (*36*), black oil glands (*23*), internal glands (*45*), gossypol cavities (*52*), and mucilage canals (*18*). According to Stanford and Viehoever (*45*), the nature of the contents of the flattened cells surrounding the cavities is dependent upon whether they are developed in darkness or exposed to light.

VEINS

The veins of *Gossypium* leaves are mostly vertically transcurrent (pl. 3, *I, L*), but some of the smaller ones are embedded (*G*). The midribs and other large veins commonly project beyond the general level of the lamina on both upper and lower surfaces of the leaf (*A–I*). The relative thickness of the major veins and of the photosynthetic areas of leaves, as well as size and shape of major veins at comparable points in the leaves of different species, shows considerable diversity within the genus. The size of both the vascular bundles of the midribs and the conducting elements composing them is likewise variable. Kuntze (*22*) reported the veins of *G. herbaceum* to be without bast, but Heim de Balsac's (*14, v. 3*) diagrammatic sketch of the midrib of this species showed groups of pericyclic fibers. The veins of most species of *Gossypium* commonly lack fibers at the outer edge of the phloem throughout the greater part of their length. However, it is not unusual to find a few bast fibers capping the phloem toward the base of the larger veins. In large veins (pl. 4, *G*) the vascular bundles or fibrovascular bundles are surrounded by parenchyma which extends to a collenchymatic layer of variable thickness underlying the epidermis, while in small veins the parenchyma extends to the epidermis. Druses, probably of calcium oxalate, are often present in the parenchyma of both large and small veins. Lysigenous cavities resembling those occurring in the mesophyll are frequently present in the parenchyma of large veins (pls. 4, *F*; 5, *A*). Mucilage canals, such as are present in the parenchyma of the veins of species of *Hibiscus* (pl. 4, *D, E*), are apparently absent in *Gossypium*. As shown in plate 4, *D* and *E*, mucilage canals may occur in vein parenchyma above as well as below the vascular bundles, where they were observed by Kuntze (*22*).

NECTARIES

Nectaries are usually a conspicuous structural feature of the leaves of *Gossypium*, but they are apparently always lacking in *G. tomentosum* and are often absent in individual leaves or plants of other species. Unlike the extrafloral nectaries described by Tyler (*47*) as characteristic of one or both sets of involucres in *Gossypium*, the leaf nectaries are considered by Lewton (*23*) to be of little value in classification.

The leaf nectaries of *Gossypium* occur on the lower surface of from one to five major veins, being most common on the midrib. Only one nectary occurs on a vein, and it is usually close to the base of the vein in most species, but in *G. kirkii* it is characteristically well above the middle of the vein. Leaf nectaries vary considerably in size within

each of the three main groups of species. Nectary size does not always correspond to leaf size, the nectaries of *G. sturtii* being very long in proportion to leaf length, and those of hybrids involving the eglandular species *G. tomentosum* generally being very small in proportion to leaf size. Often the nectaries on major lateral veins are smaller than those on the midrib of the same leaf. Leaf nectaries likewise show wide variation in size in the close relatives of *Gossypium*, those of *Shantzia* being noteworthy because of their great length.

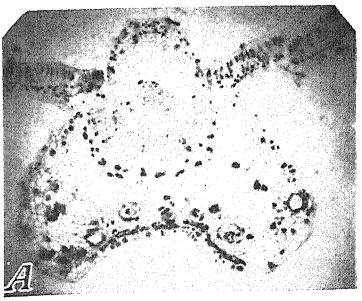
The leaf nectaries of *Gossypium* are most commonly rounded-triangular in outline, but may be nearly circular, elliptical, elongate-quadrangular, or sagittate, the shape showing no correlation with the main divisions of the genus. Cross sections taken at approximately the middle of nectaries (pl. 5, *A–I*) show that the extent of depression of the glandular surface, the size and shape of glandular hairs, and the contour of the sides of the nectaries are also variable.

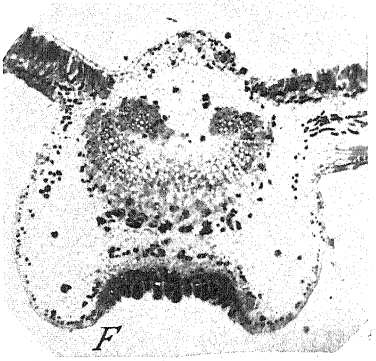
Janda (*18*) recently classified the extrafloral nectaries of *Gossypium*, *Thespesia trilobata*, and *Cienfuegosia* as depressed nectaries, or nectaries with the secretory surface below the surrounding surface. In contrast, the nectaries of *Hibiscus rosa-sinensis*, *H. schizopetalus*, *H. archeri*, *H. syriacus*, and *Thespesia populnea* were classified as surface nectaries, or nectaries with the secretory surface lying at or near the same level as the surrounding epidermis; and the nectaries of *Kydia*, *Urena*, *Decaschistia*, *Dicellostyles*, *Julostylis*, and some species of *Hibiscus* were described as hollow nectaries, or nectaries with the secreting surface covering a cavity which has a small, slitlike orifice. Since Janda listed different species of *Thespesia* and *Hibiscus* as having different types of nectaries, it is not surprising that cross sections of some *Gossypium* nectaries (pl. 5, *C, D*) show that they might well be described as surface nectaries rather than depressed nectaries.

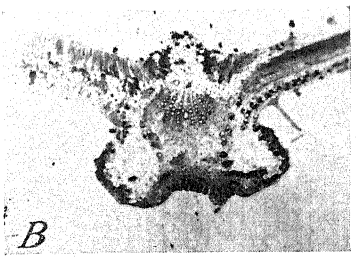
The ontogeny of the nectaries of cotton leaves has been traced by Schwendt (*39, pp. 254–256*) in *Gossypium brasiliense* and by Reed (*31*) in *G. hirsutum*.. The histology of mature leaf nectaries of *G. brasiliense*, *G. herbaceum*, *G. davidsonii*, and *G. microcarpum* Tod. was compared by Schwendt (*39*), and the structure of leaf nectaries and involucral nectaries of *G. purpurascens* was compared by Janda (*18*). Although the leaf nectaries of different species of *Gossypium* vary in such characteristics as the size and number of glandular hairs, the number of simple or stellate hairs, and the size of cells composing their glandular and subglandular tissue, their general structure shows a marked similarity in all species. As in extrafloral nectaries discussed by Solereder (*43*), the upper, expanded portion of the glandular hairs of *Gossypium* nectaries is generally more strongly developed than that of capitate hairs of similar structure found elsewhere on the same plant (pl. 4, *G, H*). Bordering the glandular hairs of a nectary, the epidermal cells are often vertically elongate and are sometimes divided horizontally. Underlying the epidermis of the nectaries are several cell layers of subglandular tissue. As shown in plate 4, *G* and *H*, the cells of the subglandular tissue are smaller than the parenchyma cells of major veins outside the nectarial region. In some nectaries, druses of calcium oxalate are particularly abundant in the subglandular tissue.

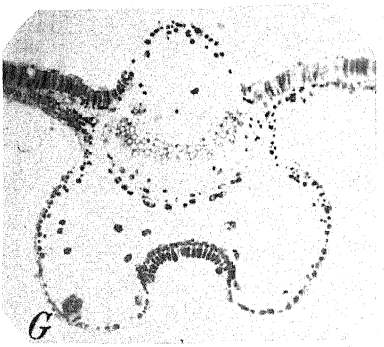
PETIOLES

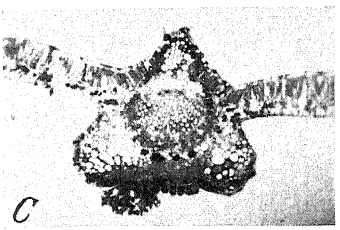
Within the genus *Gossypium* petioles of mature leaves vary greatly in size. According to Afzal (*1*) the petiole attains its full size at the

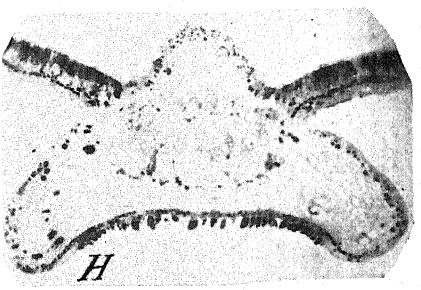


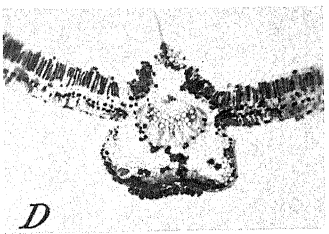


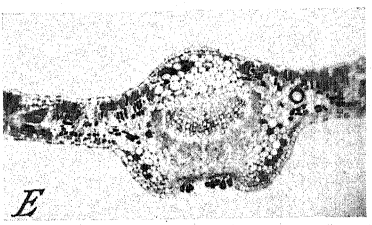


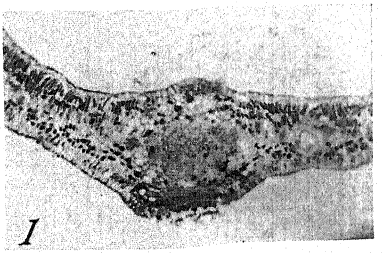




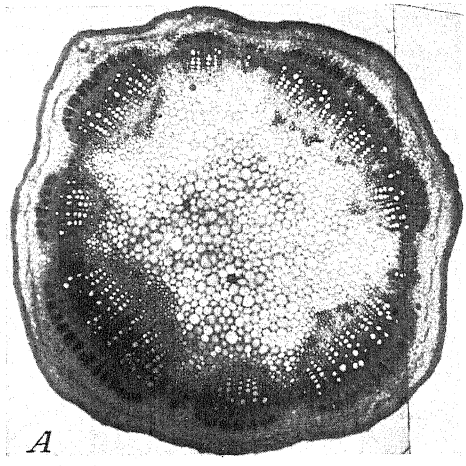


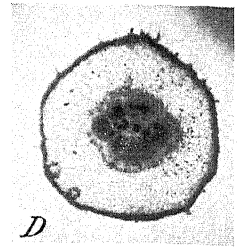


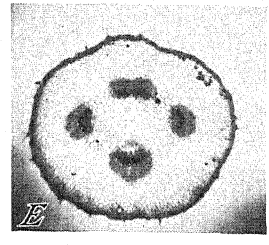


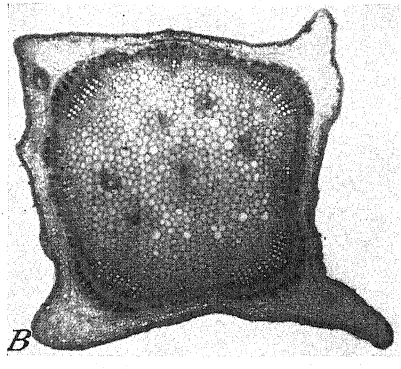


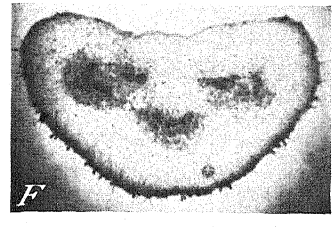
Cross sections of *Gossypium* leaf nectaries. × 25. *A, G. klotzschianum; B, G. nanking; C, G. herbaceum D, G. africanum; E, G. sturtii; F, G. darwinii; G, G. peruvianum; H, G. thurberi; I, G. harknessii.*



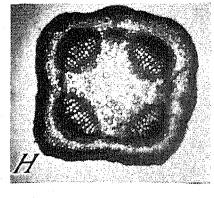


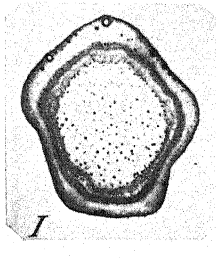


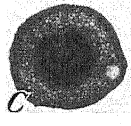


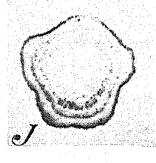


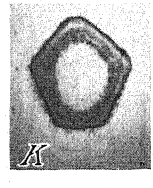


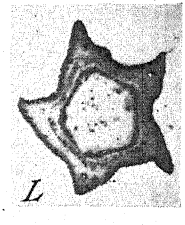


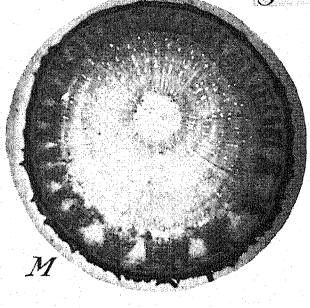


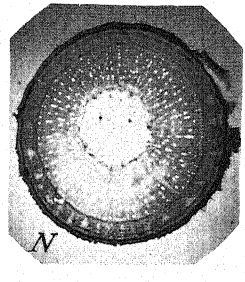


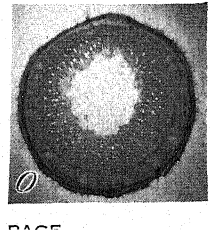












FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE

same time as the lamina, and there is a high correlation between length of petiole and length of midrib. There is also a general tendency for long petioles to be of greater diameter than short petioles, but individual exceptions to this generalization are of fairly common occurrence. The shape of a petiole in cross section depends considerably on the point in the petiole at which the section is taken (pl. 6, *D*, *E*, *F*). An indication of the range in size and shape of the midsections of petioles of *Gossypium* may be gained from plate 6, *A*, *B*, *C*, *E*, *G*, and *H*.

The epidermis of the petiole bears a marked resemblance to that of the major veins. The various types of epidermal hairs are well developed on the petiole and are often more conspicuous than on the midrib. Underlying the epidermis is a multicellular layer of collenchyma such as Plitt (*29*) described as characteristic of malvaceous petioles. The prominent ridges of the petioles of *G. kirkii* (pl. 6, *B*), which give them their quadrangular outline in cross section, are composed mostly of this tissue. Between the collenchyma and the well-developed fibers capping the phloem of the vascular bundles is a parenchymatic layer several cells deep. Oblate or spherical lysigenous cavities similar to those occurring in the parenchyma of veins are usually of scattered occurrence in this parenchymatic layer (*A–H*). At the midpoint of the petiole the fibrovascular system may take the form of a ring (*A*, *B*), an arc open at the top (*C*), or isolated bundles arranged in a ring (*E*). This diversity in arrangement of the vascular tissues of the petioles of *Gossypium* is greater than is indicated for the family Malvaceae by Dumont (*8*), Plitt (*29*), or Solereder (*43*).

While many species of *Gossypium* and related genera have dimorphic leaves (*3*), *G. thurberi* is notable for its diversity of leaf form. In this species the arrangement of the vascular tissues in cross sections taken at the midpoints of petioles of an entire leaf (pl. 6, *G*) and of leaves with two, three, four, and five lobes (*H*) showed but slight differences.

The central parenchyma of a petiole is usually homogeneous, although some of its cells frequently are conspicuous because of their dark-brownish contents (pl. 6, *A*). The central parenchyma of the petioles of the aberrant *G. kirkii* differs from that of the typical species of *Gossypium* examined in that it occasionally includes a few small medullary bundles and generally contains scattered spherical or oblate lysigenous cavities similar to those found in the lamina and in the cortex of the petiole (*B*). The occurrence of these cavities in the central parenchyma of the petiole of *G. kirkii* is of significance since Lewton (*23*) has pointed out that the distribution as well as the presence or absence of such cavities should be regarded as an anatomical character of value in classifying the Hibisceae.

EXPLANATORY LEGEND FOR PLATE 6.

Cross sections of *Gossypium* petioles and stems. *A–H*, Petioles (× 15): *A*, *B*, *C*, *E*, *G*, and *H*, Sections taken at approximately midpoint of petiole; *D*, section taken near top of petiole; *F*, section taken near base of petiole; *A*, *G. brasiliense*; *B*, *G. kirkii*; *C*, *G. armourianum*; *D–F*, *G. nanking*; *G*, *H*, *G. thurberi*. *I–O*, Stems (× 7½): *I–L*, very young stems; *M–O*, older stems; *I* and *M*, *G. purpurascens*; *J* and *N*, *G. arboreum*; *K* and *O*, *G. davidsonii*; *L*, *G. kirkii*.

STEM ANATOMY

SHAPE OF THE STEM AND ARRANGEMENT OF THE TISSUES

The stems of all species of *Gossypium* vary in cross-sectional shape at different stages in their development. As shown in plate 6, *I–L*, sections taken near the apices of the stems of Old World cottons, an American wild cotton, and a cultivated American cotton are angular and are generally inclined to be pentagonal in form. In contrast, sections of older stems (*M–O*) are rounded, often nearly circular, but sometimes broadly elliptic in outline.

The arrangement of the tissues in stems of *Gossypium* may be seen in plate 6, *I–O*, and plate 7, *A*, *B*, *C*, *E*. In centripetal order the primary permanent tissues (pl. 7, *B*) are epidermis, cortex merged with pericycle, phloem, cambium, xylem, and pith. In older stems the epidermis is underlain by periderm (*C*), which later replaces it (*E*).

EPIDERMIS AND PERIDERM

The epidermis of the stem is quite similar to that of the petiole The characteristic types of epidermal hairs are usually well developed near the apex of a stem but are less numerous where the epidermis is older. In a given plant, the capitate hairs near the apex of a stem are sometimes considerably larger than those over the green portions of the leaf blades, and approach those of the nectaries in size. A two-layered epidermis is present in the stems of some relatives of *Gossypium*, notably *Hibiscus splendens* (*24*) and *Alyogyne hakeaefolia* (pl. 7, *D*).

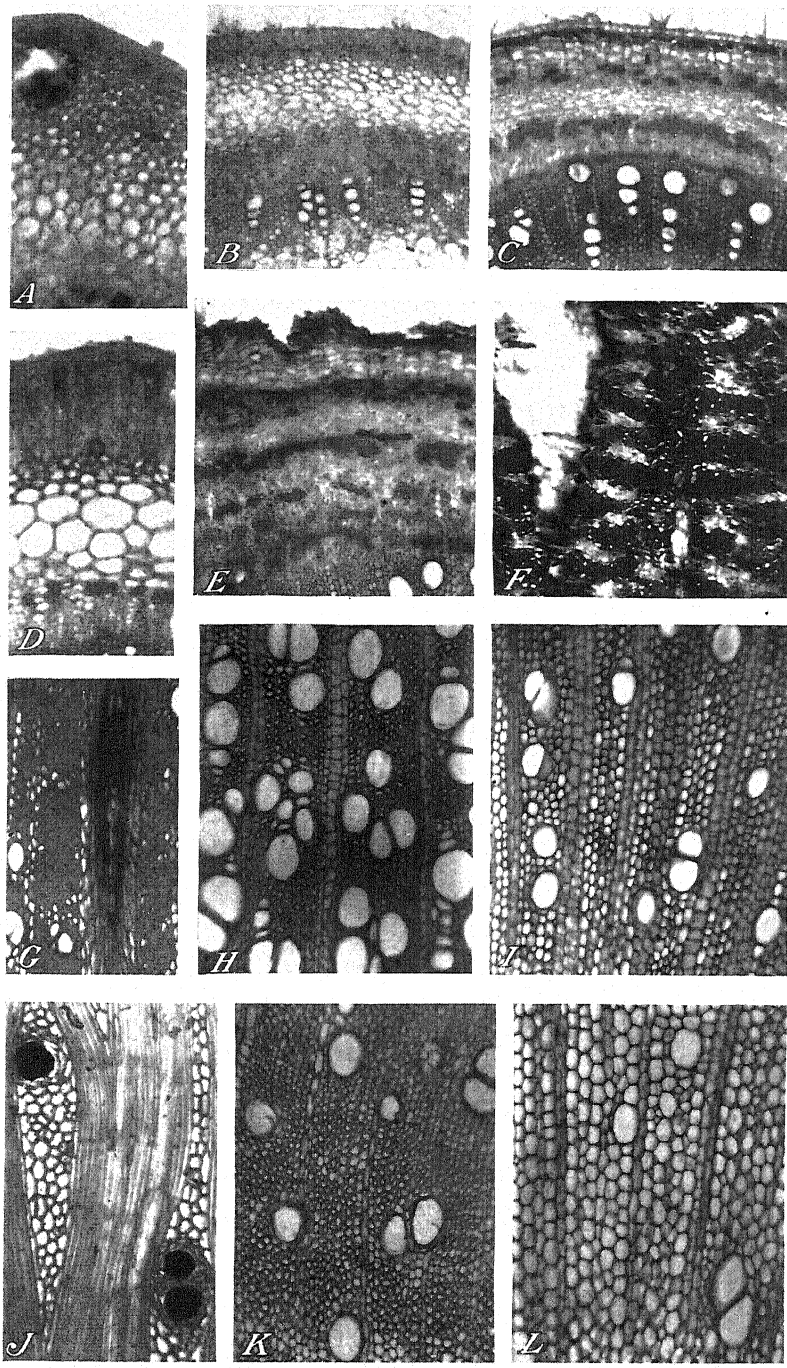
Periderm formation in the stems of *Gossypium* usually begins before the end of the first growing season. In numerous species of *Gossypium* the phellogen that gives rise to the periderm (pl. 7, *C*) forms in the outermost layer of cortical cells. A subepidermal phellogen is also characteristic of *Erioxylum aridum* and, according to Moeller (*27*), of *Hibiscus syriacus* L. and *Lavatera olbia* L. Solereder (*43*) reported that phellogen of epidermal origin is much less common in the Malvaceae, being known to occur only in the Ureneae, *Sida pulchella*, and most species of *Hibiscus*. At different ages the periderm of any given species is of rather different appearance. Different stages in the development of periderm in stems of *G. harknessii* are shown in plate 7, *C* and *E*. As has been reported by Kuntze (*22*), the phellem cells of most species of *Gossypium* often have brownish contents. The comparatively rare occurrence of oblate or spherical lysigenous cavities in the phelloderm of *Gossypium* stems has been discussed by Stanford and Viehoever (*45*).

CORTEX AND PERICYCLE

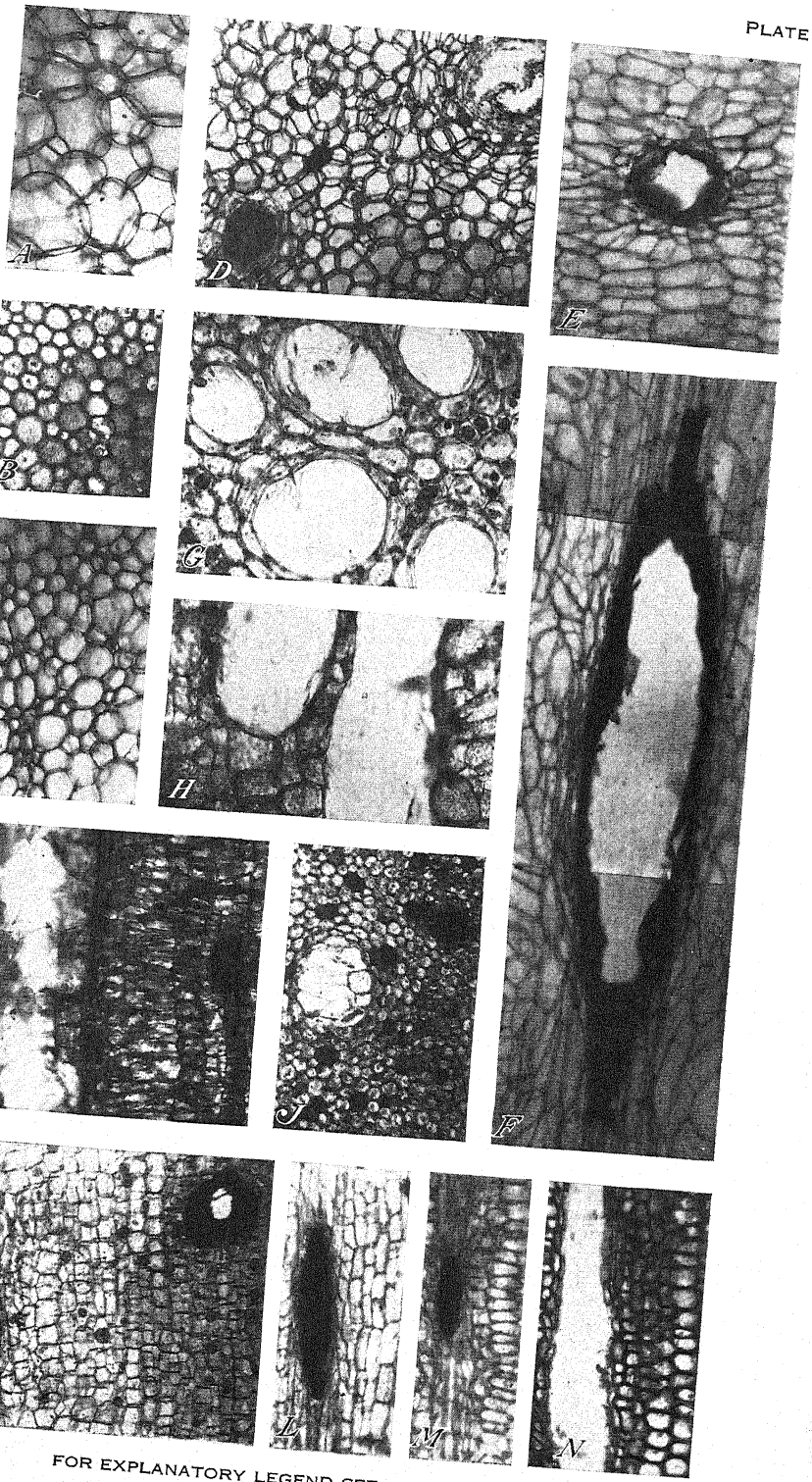
The general occurrence in the primary cortex of the stems of Malvaceae of an outer and an inner parenchymatous zone separated by a collenchymatous zone was reported by Solereder (*43*). These three

EXPLANATORY LEGEND FOR PLATE 7.

Stem sections. × 60. *A* and *D*, Stem epidermis and cortex: *A*, *Gossypium brasiliense*; *D*, *Alyogyne hakeaefolia*. *B*, *C*, *E*, *F*, *G. harknessii*; *B*, Young stem showing primary permanent tissues; *C*, somewhat older stem showing origin of periderm; *E*, somewhat older stem than *C*, showing replacement of epidermis by periderm and early stage in stratification of phloem; *F*, stratified phloem in old stem. *G* and *J*, *G. armourianum*, xylem showing lysigenous cavities in rays: *G*, Cross section; *J*, tangential section. *H*, *I*, *K*, and *L*, Cross sections of xylem showing differences in size and distribution of xylem elements within species: *H* and *I*, *G. africanum*; *K* and *L*, *G. hirsutum* var. Acala.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

zones are usually present in the cortex of stems of *Gossypium*, but, as shown in plate 7, *A* and *B*, they vary considerably in thickness and in the size and character of the cells composing them. Even greater variation in the characteristics of the three cortical zones is found in the Hibisceae. This may be seen by comparing the cortex of the stem of *Alyogyne hakeaefolia* (pl. 7, *D*) with that of species of *Gossypium*. Oblate or spherical lysigenous cavities, similar to those described in the leaves, were present in the cortex of all species of *Gossypium* examined. They occur in all three cortical zones, but are perhaps more common in the outer parenchymatous zone and the collenchymatous zone (*A*) than in the inner parenchymatous zone. This distribution of lysigenous cavities is in contrast to that of mucilage canals in related genera, which are reported (*8*) to occur in the outer parenchymatous zone only in the Eumalveae and are usually formed in the inner parenchymatous zone.

In *Gossypium* and related genera (pl. 7, *D*), pericyclic fibers are developed during the first year's growth of the stem. They show some differences in diameter and thickness of cell wall in stems of different ages (*B, C*), and perhaps among different species. They adjoin the phloem in groups of variable size (*B, C, E*).

PHLOEM AND XYLEM

Moeller (*27*) and Solereder (*43*) have called attention to the characteristic shape of the phloem portion of vascular bundles in the Malvaceae. As shown in plate 6, *M–O*, the phloem between the primary rays is considerably broader adjoining the cambium than at its outer edge, while in contrast the rays are broadened outward. Although Dumont (*8*) considered stratification of the phloem as one of the most characteristic features of the Malvaceae, he reported that this characteristic is obscure in the Hibisceae, and particularly so in the Gossypieae, because of the sparsity of phloem fibers, these being entirely absent in *Cienfugosia* (*Fugosia*). In the various species of *Gossypium* examined, the phloem is distinctly stratified (pls. 6, *M;* 7, *E, F*) in all but very young stems. The groups of fibers are, however, more conspicuous in some specimens than in others. The fairly common occurrence of oblate or spherical lysigenous cavities in the phloem rays of *Gossypium* has been noted by Stanford and Viehoever (*45*). According to Hawkins, Matlock, and Hobart (*13*), phloem growth in Acala cotton was stimulated by increased soil moisture, but to a lesser extent than was the xylem.

The xylem of the Hibisceae was described by Dumont (*8*) as very parenchymatous, with few but very large vessels. Webber's (*50, 51, 52*) descriptions of malvaceous woods indicate much more structural diversity in the woods of this tribe. A difference in porosity or compactness of the wood of Old World and New World cottons was reported by Zaitzev (*54*). As shown in plate 7, *H, I, K, L*, woods of both Old World and New World cottons often show wide variation

Sections of pith. × 60. *A, B, C*, Cross sections of pith without lysigenous cavities or mucilage canals: *A, Gossypium peruvianum; B, G. armourianum; C, G. neglectum. D, E, F, G. kirkii*, showing variation in size and form of lysigenous cavities in pith: *D*, Cross section; *E* and *F*, longitudinal sections. *G* and *H, Hibiscus brackenridgei* showing mucilage canals in pith: *G*, Cross section; *H*, longitudinal section. *I* and *J, Thespesia lamps*, pith showing nearly spherical lysigenous cavities with dark contents and mucilage canals: *I*, Longitudinal section; *J*, cross section. *K, Kokia drynarioides*, longitudinal section showing lysigenous cavities of different forms in pith. *L, Eriodylum aridum*, longitudinal section showing elongated lysigenous cavity in pith. *M* and *N, Shantzia garckeana*, longitudinal sections of pith: *M*, Showing lysigenous cavity with dark contents; *N*, showing mucilage canal.

in size of the xylem elements and abundance of vessels. Although Stanford and Viehoever (*45*) failed to find oblate or spherical lysigenous cavities in the xylem of *Gossypium*, they were noted by Webber (*52*) in the xylem rays of *G. mexicanum*, *G. morrilli*, *G. peruvianum*, and *G. schottii*. They are also of frequent occurrence in the xylem rays of *G. armourianum* (*G*, *J*). As reported by Dumont (*8*), reddish-brown contents are often abundant in the ray cells and wood parenchyma of the xylem of *Gossypium*.

PITH

The pith of *Gossypium* is entirely parenchymatous, lacking the stone cells and sclerenchyma fibers reported by Solereder (*43*) as characteristic of some Malvaceae. Within a single species the cells vary considerably in size, wall thickness, and contents at different stages in their development. Comparable differences occur between mature pith cells of different species (pl. 8, *A*, *B*, *C*). The pith of *Alyogyne hakeaefolia* resembles that of *Gossypium*, and according to Mentovich (*26*) the pith is homogeneous in *Hibiscus syriacus* and *H. sinensis*. Although scattered cells with yellowish or brownish contents are common in all species of *Gossypium*, lysigenous cavities with brownish contents were observed within the genus only in the very aberrant *G. kirkii* (pl. 8, *D*, *E*, *F*). Similar cavities occur also in the pith of the related species *Erioxylum aridum* (*L*), *Shantzia garckeana* (*M*), *Kokia drynarioides* (*K*), and *Thespesia lampas* (*I*, *J*). Although the lysigenous cavities in the pith of *G. kirkii* and *Erioxylum aridum* are often spherical or nearly so (*E*), they are at times much more vertically elongate (*F*, *L*) than those observed elsewhere in the stems and leaves of *Gossypium* and related genera. Some of these elongate cavities might be regarded as short ducts or canals. They are, however, considerably shorter than most of the mucilage canals occurring in the pith of *Lagunaria patersonii*, *Hibiscus brackenridgei* (*G*, *H*), *Thespesia lampas* (*I*, *J*), *Kokia drynarioides*, and *Shantzia garckeana* (*N*). According to Dumont (*8*), who discussed the distribution of mucilage canals in the Malvaceae, such ducts are abundant in the Eumalveae, Sideae, and Malopeae, but comparatively rare in the Hibisceae. Within the latter tribe he observed them in *Hibiscus splendens*, *H. cameroni*, and *H. palustris*.

DISCUSSION

A histological examination of the leaves and stems of Old World cottons ($n=13$), American wild cottons ($n=13$), and New World cultivated cottons ($n=26$), has shown that within each of these groups there is considerable variation in certain structural characteristics that have hitherto been suggested as valuable in distinguishing between Old World and New World cottons. It seems probable that some of the reports of distinctive characters for the different groups were based on superficial examination of specimens or on the examination of too limited a collection of specimens to warrant the generalizations that were made.

The type of pubescence has been suggested by Zaitzev (*54*), who excludes the wild American cottons with 13 pairs of chromosomes from the genus *Gossypium*, as a means of distinguishing between Old World and New World cottons. He listed among distinctive morpho-

logical characteristics of the two groups the universal absence in New World cottons of a "two-layered pubescence,—i. e., pubescence of long and short hairs" and the occasional presence of this character in Old World species. This distinction is very misleading since it is applicable only to macroscopic stellate hairs and simple hairs and not to minute capitate or glandular hairs, which appear to be present throughout the genus, even on leaves often described as entirely glabrous. When the presence of the short capitate hairs is considered, the pubescence of New World cottons is seen to be always two-layered and that of the Old World cottons sometimes three-layered.

Aside from the nature of the pubescence, no epidermal character has been found that may be used to distinguished any of the main groups of *Gossypium*. Although there is considerable diversity in epidermal structure within the genus, none of the structural features, such as relative number of stomata on the upper and lower surfaces of the leaf, size and shape of stomata, level of the guard cells in relation to that of ordinary epidermal cells, or relative size and shape of the ordinary epidermal cells on the upper and lower leaf surfaces, is of value in delimiting the main groups of species, as there is considerable variation in each of these characteristics within every group.

Although Magitt and Magitt (*25*) did not include American wild cottons in their study, they reported the distribution of palisade tissue as differing in the leaves of Old World and New World cottons. Nevertheless, species with bifacial leaves and with subcentric to centric leaf structure occur within each of the three groups. The distribution of palisade tissue in the leaves of American wild cottons is of particular interest in view of Skovsted's (*42*) report on the relationships within the group. He considered *Erioxylum aridum*, which he renamed *Gossypium aridum*, *G. thurberi* (*20*), *G. armourianum*, and *G. harknessii* to be more closely related to one another than to *G. davidsonii* and *G. klotzschianum*. While the mesophyll of *G. armourianum* resembles that of *G. harknessii* in having well-developed palisade tissue on both sides of the leaf, the mesophyll of *G. thurberi* and *Erioxylum aridum*, which are also natives of dry areas, resembles that of *G. davidsonii* and *G. klotzschianum* in having spongy parenchyma adjacent to the lower epidermis of the leaf.

The shape of the stems in cross section is one of the characters enumerated by Zaitzev (*54*) as useful in distinguishing between Old World and New World cottons. He states that the cross section of the stem is circular in cottons of the Old World group, but oval or angular in New World cottons. This statement was evidently based on an examination of stems of different ages. As shown in plate 6, *I–L*, the stems of cottons belonging to both groups discussed by Zaitzev, and of American wild cottons as well, are angular, usually pentagonal in cross section near their apices. As the stems grow older they lose their angularity. Cross sections of 1-year-old and older stems are usually oval or nearly circular, and are often more nearly circular in New World than in Old World species. A "square stem" is listed by Skovsted (*41*) among the characteristics that distinguish *Gossypium kirkii*, the type of his genus *Gossypioides*, from most of the species of *Gossypium*. As shown in plate 6, *L*, very young stems of *G. kirkii* are star-shaped in cross section. As the stems grow older they become less noticeably ridged and nearly pentagonal in

cross-sectional outline. In view of the widespread occurrence of a similar shape in the sections of young stems of other species of *Gossypium*, the stem shape of *G. kirkii* is less peculiar than Skovsted considered it to be. It should, however, be pointed out that the stems of *G. kirkii* retain their angularity longer than those of more typical species of *Gossypium*.

In view of the reported (*43*) epidermal origin of phellogen in most species of *Hibiscus* and the subepidermal origin of phellogen in *Gossypium*, determination of the place of origin of phellogen in *G. kirkii* might have some taxonomic bearing. Skovsted (*41*) has shown that this species in some respects resembles *Hibiscus* rather than *Gossypium*. Unfortunately the origin of the phellogen cannot be determined in material of this species now available.

Zaitzev (*54*) considered the xylem structure of Old World and New World cottons as sufficiently distinct to be useful in distinguishing between the two groups. He described the wood of Old World cottons as "compact" and that of New World cottons as "porous." This terminology is confusing in view of the technical terms used in describing wood (*17*). The woods of all species of *Gossypium* are "porous" in the sense that they have pores, i. e. vessels in cross section. Accordingly Zaitzev's (*54*) descriptions of differences in porosity of the woods of different groups of *Gossypium* might be interpreted to refer to differences in the number of vessels only, but it seems probable that they refer instead to differences in texture, which is dependent on the size of wood elements. Regardless of whether difference in vessel number or difference in size of various wood elements was meant by Zaitzev, his distinction between the woods of Old World and New World cottons is not valid. As shown in plate 7, *H*, *I*, *K*, *L*, the woods of both New World and Old World cottons are diffuse-porous, but the number of pores in a unit of area and the size of the various wood elements are often highly variable in the same stem. Hawkins, Matlock, and Hobart (*13*) have shown that the size of the wood elements and the thickness of their walls in Acala cotton (*G. hirsutum*) are influenced by available soil moisture.

The presence and distribution in the Hibisceae of oblate or spherical lysigenous cavities filled with brownish contents was regarded by Lewton (*23*) as a valuable characteristic in classifying this group. It is therefore noteworthy that such cavities may occur in xylem rays, central parenchyma of petioles, and pith of stems, in addition to the tissues in which their occurrence was reported by Stanford and Viehoever (*45*). In the pith of *G. kirkii*, in addition to the common oblate or spherical lysigenous cavities (pl. 8, *E*), various transitional forms to short, canallike cavities with dark contents occur (*F*). Both typical oblate or spherical cavities and typical mucilage canals occur in the pith of *Kokia*, *Shantzia*, and *Thespesia*. Dumont's (*8*) distinction between Euhibisceae with mucilage canals and Gossypieae with spherical cavities is therefore invalid.

The presence of lysigenous cavities in the pith of the stems and petioles of *G. kirkii* and their absence in all other species of *Gossypium* examined is of significance, since, for cytological reasons, the inclusion of this species in the genus *Gossypium* has been questioned (*41*). This characteristic may therefore be considered as further grounds for excluding this species from the genus *Gossypium*. Since Skovsted

(*41*) has noted the resemblance of *G. kirkii* to the *Hibiscus* subtribe as well as to the *Gossypium* subtribe, the presence in this species of both spherical lysigenous cavities such as are common in *Gossypium* and elongated cavities approaching the form common in *Hibiscus* is of interest.

Erioxylum aridum has been referred to the genus *Gossypium* on cytological grounds (*41*, *42*). It should therefore be noted that lysigenous cavities such as occur in the pith of *Erioxylum* (pl. 8, *L*) are not characteristic of the pith of *Gossypium*, although they occur in other tissues in the latter genus.

SUMMARY

The descriptions of leaf and stem structure of *Gossypium* presented in this paper are based on a histological examination of the leaves and stems of 12 Old World species, 5 wild American species, 10 cultivated or semiwild American species, 2 doubtful species, and 9 species belonging to related genera.

There is more anatomical variation within each of these three groups, and less between the several groups, than previous descriptions indicated.

Since the distribution of spherical or oblate lysigenous cavities has been thought to be of value in classifying the Hibisceae, their hitherto unreported occurrence in the xylem rays of *G. armourianum* and in the pith of *G. kirkii*, *Erioxylum aridum*, *Kokia drynarioides*, *Shantzia garckeana*, and *Thespesia lampas* is significant.

The presence of both spherical lysigenous cavities and elongate mucilage canals in the pith of *Kokia*, *Thespesia*, and *Shantzia* is of interest since the type of cavity present in either cortex or pith has been suggested as a means of distinguishing between the subtribes Euhibisceae and Gossypieae of the tribe Hibisceae.

The occurrence of spherical to elongate lysigenous cavities in the pith of *G. kirkii* may be considered as an additional reason for excluding this species from the genus *Gossypium*, since no such cavities were observed in the pith of typical species of *Gossypium*.

In regard to Skovsted's proposed inclusion of *Erioxylum aridum* in the genus *Gossypium*, it should be pointed out that this species differs from all typical species of that genus that were examined in having lysigenous cavities in its pith.

LITERATURE CITED

(1) Afzal, Mohammad.
1933. development and shedding of leaves of cotton. Indian Jour. Agr. Sci. 3: 97–115, illus.

(2) Bachmann, Otto.
1886. untersuchungen über die systematische bedeutung der schildhaare. Flora [Jena] 69: [387]–400, [403]–415, 428–448, illus.

(3) Cook, O. F.
1911. dimorphic leaves of cotton and allied plants in relation to heredity. U. S. Bur. Plant Indus. Bull. 221, 59 pp., illus.

(4) ——— and Mead, Rowland M.
1911. arrangement of parts in the cotton plant. U. S. Bur. Plant Indus. Bul. 222, 26 pp., illus.

(5) Dantas, Garibaldi.
1936. o algodoeiro sua estructura. Algodão No. 23, pp. 32–36, illus.

(6) DISCHENDORFER, OTTO.
1925. ZUR KENNTNIS DER BAUMWOLLFASER. Angew. Bot. 7: [57]–73, illus. [Original not seen.]
(7) DOAK, CLIFTON C.
1937. THE PISTIL ANATOMY OF COTTON AS RELATED TO EXPERIMENTAL CONTROL OF FERTILIZATION UNDER VARIED CONDITIONS OF POLLINATION. Amer. Jour. Bot. 24: 187–194, illus.
(8) DUMONT, ARTHUR.
1888. RECHERCHES SUR L'ANATOMIE COMPARÉE DES MALVACÉES, BOMBACÉES, TILIACÉES, STERCULIACÉES. Ann. Sci. Nat., Bot. (7) 6: 129–246, illus.
(9) FLATTERS, ABRAHAM.
1906. THE COTTON PLANT: ITS DEVELOPMENT AND STRUCTURE AND THE EVOLUTION AND STRUCTURE OF THE COTTON FIBRE. 92 pp., illus. London.
(10) GORE, ULYS ROY.
1935. MORPHOGENETIC STUDIES ON THE INFLORESCENCE OF COTTON. Bot. Gaz. 97: 118–138, illus.
(11) ——— and TAUBENHAUS, J. J.
1931. ANATOMY OF NORMAL AND ACID-INJURED COTTON ROOTS. Bot. Gaz. 92: 436–441, illus.
(12) HARLAND, S. C., and ATTECK, O. S.
1931. INTERGENERIC HYBRIDS BETWEEN GOSSYPIUM AND THURBERIA. Amer. Nat. 65: 380–382.
(13) HAWKINS, R. S., MATLOCK, R. L., and HOBART, CHARLES.
1933. PHYSIOLOGICAL FACTORS AFFECTING THE FRUITING OF COTTON WITH SPECIAL REFERENCE TO BOLL SHEDDING. Ariz. Agr. Expt. Sta. Tech. Bull. 46, pp. [361]–407, illus.
(14) HEIM DE BALSAC, F., and HEIM DE BALSAC, H.
1926–29. CONTRIBUTION À L'ÉTUDE BOTANIQUE DES COTONNIERS. ATLAS ICONOGRAPHIQUE DU COTONNIER. ORGANISATION-STRUCTURE. Coton et Cult. Coton. 1 (fasc. 3): [79]–86, 163–169, 211–216, illus., 1926; 2: 62, 64, illus., 1927; 3: 67–72, 177–184, 251–254, illus., 1928; 4: 163–166, illus. 1929.
(15) HÖHNEL, FRANZ R. V.
1881. ANATOMISCHE UNTERSUCHUNGEN ÜBER EINIGE SECRETIONSORGANE DER PFLANZEN. Sitzber. Akad. Wiss. Wien, Math. Naturw. Kl. 84: 565–603, illus.
(16) HUBBARD, J. W.
1931. FARM STUDY OF THE COTTON PLANT. U. S. Dept. Agr. Farmers' Bull. 1661, 18 pp., illus.
(17) INTERNATIONAL ASSOCIATION OF WOOD ANATOMISTS, COMMITTEE ON NOMENCLATURE.
1933. GLOSSARY OF TERMS USED IN DESCRIBING WOODS. Trop. Woods 36: 1–12.
(18) JANDA, CHARLOTTE.
1937. DIE EXTRANUPTIALEN NEKTARIEN DER MALVACEEN. Oesterr. Bot. Ztschr. 86: [81]–130, illus.
(19) KEARNEY, THOMAS H.
1934. AMERICAN WILD COTTONS WITH THIRTEEN CHROMOSOMES. Jour. Heredity 25: 305–312, illus.
(20) ———
1937. INGENHOUZIA AND THURBERIA. Amer. Jour. Bot. 24: 298–300.
(21) KERR, THOMAS.
1937. THE STRUCTURE OF THE GROWTH RINGS IN THE SECONDARY WALL OF THE COTTON HAIR. Protoplasma 27: [229]–241, illus.
(22) KUNTZE, GEORG.
1891. BEITRÄGE ZUR VERGLEICHENDEN ANATOMIE DER MALVACEEN. Bot. Centbl. 45: [161]–168, [197]–202, [229]–234, [261]–268, [293]–299, [325]–329, illus.
(23) LEWTON, FREDERICK L.
1925. THE VALUE OF CERTAIN ANATOMICAL CHARACTERS IN CLASSIFYING THE HIBISCEAE. Jour. Wash. Acad. Sci. 15: 165–172.
(24) LINSBAUER, K.
1930. DIE EPIDERMIS. 283 pp., illus. Berlin. *In* Linsbauer, K., Handbuch der Pflanzenanatomie, Abt. 1, Teil 2, Histologie, Bd. 4.

(25) MAGITT, M., and MAGITT, E.
1929. STUDIEN UEBER DIE ANATOMIE DES BAUMWOLLENSTRAUCHS. II. DAS PALISSADENPARENCHYM IM BLATT DES BAUMWOLLENSTRAUCHS. Jour. Soc. Bot. Russie 14: 191–198, illus. [In Russian. German summary, pp. 197–198.]

(26) MENTOVICH, FERENC VON.
1886. ZUR HISTOLOGIE DES PFLANZLICHEN MARKES, MIT BESONDERER RÜCKSICHT AUF DIE DIKOTYLEN. Reviewed in Bot. Centbl. 26: 67–75.

(27) MOELLER, JOSEPH.
1882. ANATOMIE DER BAUMRINDEN. 447 pp., illus. Berlin.

(28) NESTLER, A.
1898. DIE SCHLEIMZELLEN DER LAUBBLÄTTER DER MALVACEEN. Oesterr. Bot. Ztschr. 48: 94–99, illus.

(29) PLITT, C.
1886. BEITRÄGE ZUR VERGLEICHENDEN ANATOMIE DES BLATTSTEILS DER DICOTYLEDONEN. Diss. Marburg 2 (20): 1–52, illus.

(30) RAUTER, J.
1872. ZUR ENTWICKLUNGSGESCHICHTE EINIGER TRICHOMGEBILDE. Denkschr. Akad. Wiss. Wien, Math. Naturw. Kl. 31 (2): 1–48, illus.

(31) REED, E. L.
1917. LEAF NECTARIES OF GOSSYPIUM. Bot. Gaz. 63: 229–231, illus.

(32) REEVES, R. G.
1935. ORIGIN OF THE FRINGE TISSUE OF THE COTTON SEED. Bot. Gaz. 97: 179–184, illus.

(33) ———
1936. COMPARATIVE ANATOMY OF THE SEEDS OF COTTONS AND OTHER MALVACEOUS PLANTS. I. MALVEAE AND URENEAE. Amer. Jour. Bot. 23: 291–296, illus.

(34) ———
1936. COMPARATIVE ANATOMY OF THE SEEDS OF COTTON AND OTHER MALVACEOUS PLANTS. II. HIBISCEAE. Amer. Jour. Bot. 23: 394–405, illus.

(35) ——— and BEASLEY, J. O.
1934. THE DEVELOPMENT OF THE COTTON EMBRYO. Jour. Agr. Research 51: 935–944, illus.

(36) ——— and BEASLEY, J. O.
1937. LOCALIZATION OF PENTOSANS IN THE RESIN GLANDS OF THE COTTON EMBRYO. Jour. Agr. Research 54: 711–718.

(37) ——— and VALLE, C. C.
1932. ANATOMY AND MICROCHEMISTRY OF THE COTTON SEED. Bot. Gaz. 93: 259–277, illus.

(38) ROBBINS, WILFRED W.
1917. THE BOTANY OF CROP PLANTS; A TEXT AND REFERENCE BOOK. 681 pp., illus. Philadelphia.

(39) SCHWENDT, EDUARD.
1907. ZUR KENNTNIS DER EXTRAFLORALEN NEKTARIEN. Bot. Centbl. Beihefte 22 (Abt. 1): 245–286, illus.

(40) SINGH, T. C. N.
1931. NOTES ON THE EARLY STAGES IN THE DEVELOPMENT OF THE COTTON-FIBRE AND THE STRUCTURE OF THE BOLL AND SEED. Ann. Bot. [London] 45: 378–380, illus.

(41) SKOVSTED, A.
1935. CHROMOSOME NUMBERS IN THE MALVACEAE. I. Jour. Genetics 31: [263]–296, illus.

(42) ———
1937. CYTOLOGICAL STUDIES IN COTTON. IV. CHROMOSOME CONJUGATION IN INTERSPECIFIC HYBRIDS. Jour. Genetics 34: 97–134, illus.

(43) SOLEREDER, HANS.
1908 SYSTEMATIC ANATOMY OF THE DICOTYLEDONS; A HANDBOOK FOR LABORATORIES OF PURE AND APPLIED BOTANY. Trans. by Boodle, L. A., and Fritsch, F. E., rev. by D. H. Scott. 2 v., illus. Oxford.

(44) SPIETH, ALDA MAY.
1933. ANATOMY OF THE TRANSITION REGION IN GOSSYPIUM. Bot. Gaz. 95: 338–347, illus.

(45) STANFORD, ERNEST E., and VIEHOEVER, ARNO.
1918. CHEMISTRY AND HISTOLOGY OF THE GLANDS OF THE COTTON PLANT, WITH NOTES ON THE OCCURRENCE OF SIMILAR GLANDS IN RELATED PLANTS. Jour. Agr. Research 13: 419–436, illus.
(46) TRÉCUL, AUGUSTE.
1866–67. DES MUCILAGES CHEZ LES MALVACÉES, LE TILLEUL, LES STERCULIACÉES, LES CACTÉES ET LES ORCHIDÉES INDIGÈNES. Adansonia 7: 248–255.
(47) TYLER, FREDERICK J.
1908. THE NECTARIES OF COTTON. U. S. Bur. Plant Indus. Bull. 131: 45–54, illus.
(48) WALLICZEK, HEINRICH.
1893. STUDIEN ÜBER DIE MEMBRANSCHLEIME VEGETATIVER ORGANE. Jahrb. Wiss. Bot. 25: [209]–277, illus.
(49) WARE, J. O.
1927. THE INHERITANCE OF RED PLANT COLOR IN COTTON. Ark. Agr. Expt. Sta. Bull. 220, 80 pp., illus.
(50) WEBBER, I. E.
1934. BEARING OF UPRIGHT RAY CELLS IN THE WOOD OF HIBISCUS MUTABILIS L. ON USAGE OF THE TERM TILE CELL. Trop. Woods 37: 9–13, illus.
(51) ———
1934. THE WOOD OF HIBISCUS TILIACEUS L. Trop. Woods 37: 14–18, illus.
(52) ———
1934. SYSTEMATIC ANATOMY OF THE WOODS OF THE MALVACEAE. Trop. Woods 38: 15–36, illus.
(53) YOUNGMAN, W., and PANDE, S. S.
1929. THE EPIDERMAL OUTGROWTHS OF THE GENERA THESPESIA AND GOSSYPIUM; A MORPHOLOGICAL STUDY THROWING SOME LIGHT UPON THE EVOLUTION OF THE HAIRS CONSTITUTING COMMERCIAL COTTON. Ann. Bot. [London] 43: [711]–740, illus.
(54) ZAITZEV, GAVRIL S.
1928. A CONTRIBUTION TO THE CLASSIFICATION OF THE GENUS GOSSYPIUM L. Turkestan Plant Breeding Sta., Tashkent, Trans. 12, 65 pp., illus. [In Russian. English summary, pp. 39–65.]

TWO MASTIGOSPORIUM LEAF SPOTS ON GRAMINEAE [1]

By RODERICK SPRAGUE [2]

Associate pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The type species of the moniliaceous genus *Mastigosporium* Riess is *M. album* Riess (*6, p. 56*)[3], which attacks a number of grasses in Wales, France, central Europe, the Union of Soviet Socialist Republics, and Scandinavia (*9, 10, 11, 15*). The fungus is distinguished by the presence of one to several tentaclelike appendages extending from the awl-shaped distal end of its hyaline, elliptical, three-(three-to-five) septate conidia (figs. 1, *J-O*, and 2).

The writer recognized a second species of *Mastigosporium*, which has nonappendaged conidia (fig. 1, *a-i*). The fungus, *M. calvum* (Ellis and Davis) comb. nov., occurs in Germany (*5*), France (*14, p. 254*), England (*16, p. 233*), Wales, Russia (*1*), Ontario, Canada, and in Wisconsin (*3, p. 98*), Montana (*4, p. 361*), and Oregon.[4]

Most of the present study deals with *Mastigosporium calvum*, as it is conveniently abundant in western Oregon. *M. album* apparently has not been found in North America.

DISTRIBUTION AND ECOLOGY OF MASTIGOSPORIUM CALVUM IN THE WESTERN UNITED STATES

The disease caused by *Mastigosporium calvum* is the most important malady of orchard grass (*Dactylis glomerata* L.) in portions of western Oregon. It is common in the northern end of the Willamette Valley from Benton County north to at least the Columbia River and east through the Columbia Gorge into Hood River County. It is prevalent on orchard grass in the coast region from Astoria, south to Lane County, which is as far south as observations were made. In commercial fields of seed grass in Clatsop County the disease is prevalent on redtop (*Agrostis alba* L.). In 1937 it was also very abundant on the locally more important Astoria colonial bent (*A. tenuis* Sibth.) in Clatsop and Tillamook Counties. Scattering infection occurs on creeping bent (*A. palustris* Huds.) in commercial bentgrass seed fields in Clatsop County and is common in pastures in Lincoln County.

The apparent susceptibility of a number of grasses is shown in table 1. These data were compiled from rod-row plots at Astoria, Oreg. All the grasses were the same age and were growing side by side in the

[1] Received for publication February 4, 1938; issued August 1938. Cooperative investigations by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Oregon and Washington Agricultural Experiment Stations. Published as Technical Paper No. 270 of the Oregon Agricultural Experiment Station.

[2] The writer is indebted to A. G. Johnson and John A. Stevenson for more than routine aid in locating material and literature; to A. G. Johnson for a critical revision of the manuscript; and to John H. Martin for translating Bondarzeva-Monteverde's article from the original Russian.

[3] Italic numbers in parentheses refer to Literature Cited, p. 299.

[4] SPRAGUE, R. A PRELIMINARY CHECK LIST OF THE PARASITIC FUNGI ON CEREALS AND OTHER GRASSES IN OREGON. U. S. Bur. Plant Indus., Plant Disease Rptr. 19: 136-186. 1935. [Mimeographed.]

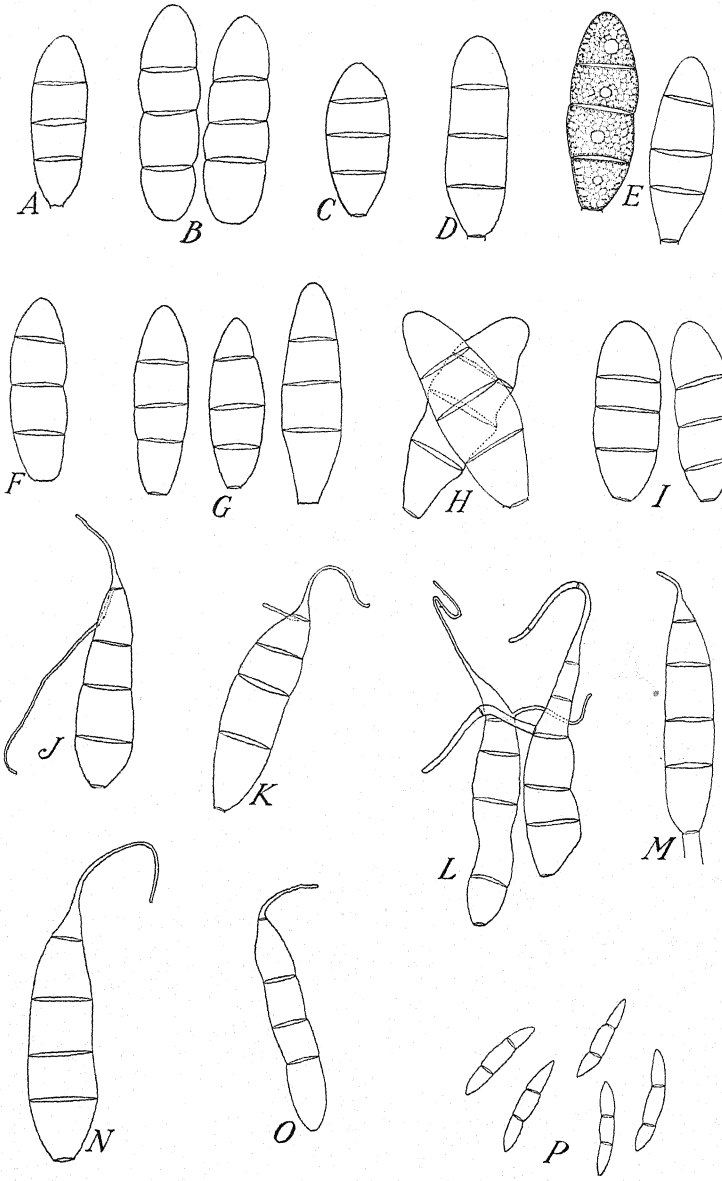


FIGURE 1.—Conidia of *Mastigosporium calvum, A-I; M. album, J-O;* and *Septogloeum athrix, P.* From following sources: *A,* On *Agrostis alba,* Astoria, Oreg., Oregon 10386; *B,* on *A. palustris,* Big Elk Creek, Lincoln County, Oreg., Oregon 8246; *C,* on *A. palustris,* Alsea Valley, Oreg., Oregon 10308; *D,* on *A. tenuis* var., Astoria, Oreg., Oregon 10585; *E,* on *Calamagrostis canadensis,* Ontario, Canada, Univ. Toronto, Crypt. Herb., 1292; *F,* on *C. canadensis,* State Line, Wis., 1903, Univ. Wis., J. J. Davis Herb.; *G,* on *C. canadensis* var. *scabra,* Glacier Park, Mont., type of *Fusoma rubricosa,* Fungi Columb., 5019; *H,* on *C. epigeios,* Germany, Sydow, Myc. Ger., 640; *I,* on *Dactylis glomerata,* Germany, Krieger, Fungi Sax., 792; *J,* on *Alopecurus pratensis,* Germany, Sydow, Myc. Ger., 542; *K,* on *Alopecurus pratensis,* Germany, Krieger, Fungi, Sax., 790A; *L,* on *A. pratensis,*[1] Germany, Krieger, Fungi Sax., 790B; *M,* on *A. pratensis,* Bohemia, Kabát and Bubák, Fungi Imp. Exs., 386; *N,* on *A. pratensis,* Russia, Buchholz et Bondarzew, Fungi Ross. Exs., Ser. A, 197; *O,* on *Deschampsia caespitosa,*[2] Germany, De Thümen, Myc. Univ., 1872; *P,* on *Calamagrostis* sp., Sweden, Eriksson, Fungi Par. Scand., 394. All × 667.

[1] This collection has a number of conidia with very robust appendages that have all the appearances of germ tubes.

[2] This collection has spores badly plasmolyzed.

rows. Infection was heavy in 1937. On redtop, for example, nearly 100 percent of the leaves had at least one spot and most of them had several. The same relative susceptibility had been noted in 1936.

In Benton, Linn, Polk, Marion, Clackamas, Multnomah, and Lincoln Counties, Oreg., the fungus recurs annually on the same

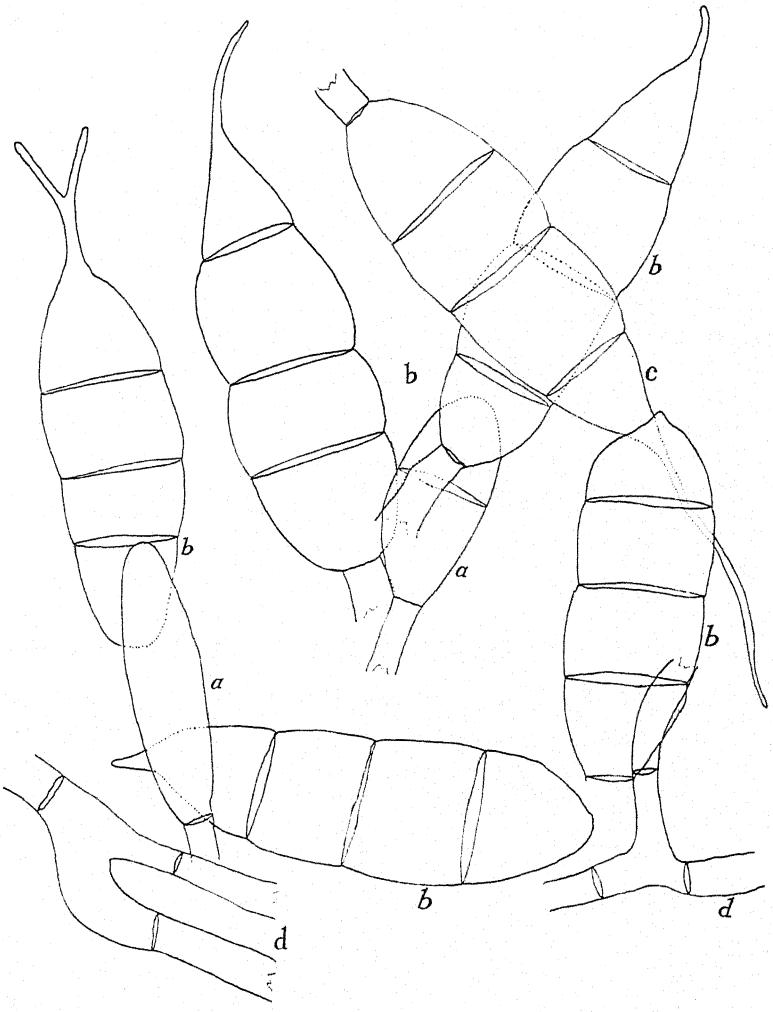


FIGURE 2.—Conidia of *Mastigosporium album* from *Alopecurus pratensis* sent by K. Sampson from Aberystwyth, Wales, April 1937, showing unplasmolyzed condition: *a*, Young conidia with attached conidiophores; *b*, maturing conidia; *c*, nearly mature conidium; *d*, fragments of hyphae. All × 1,375.

plants of orchard grass. The disease very largely disappears during the summer, but after rains start in the autumn it appears again following periods of growing weather. The disease has been most evident during February, March, and April. In the very humid coastal counties of Lincoln, Tillamook, and Clatsop the disease has continued to develop later in the spring than elsewhere, the lateness of development depending largely on the seasonal prevalence of rains. The fungus is evidently soon checked by low humidity.

TABLE 1.—*Relative susceptibility to Mastigosporium calvum of certain grasses grown under humid field conditions in the plots at the John Jacob Astor Experiment Station, Astoria, Oreg., as indicated by observations made May 12, 1937*

Host		Relative susceptibility
Technical name	Common name	
Agrostis alba L.	Redtop	Completely susceptible.
A. canina L.	Velvet bent	Susceptible.
A. palustris Huds.	Creeping bent	Do.
A. stolonifera L.	do	Moderately susceptible.
A. tenuis Sibth.	Astoria colonial bent	Very susceptible.
A. tenuis Sibth.	Rhode Island colonial bent	Susceptible to very susceptible.
A. tenuis var.	Highland colonial bent	Do.
A. verticillata Vill.	Water bent	Slightly resistant.
Alopecurus pratensis L.	Meadow foxtail	Apparently immune.
Arrhenatherum elatius (L.) Mert. and Koch.	Tall oatgrass	Do.
Dactylis glomerata L.	Orchard grass	Susceptible.
Lolium perenne L.	Perennial ryegrass	Apparently immune.
Phleum pratense L.	Timothy	Do.

Fusoma rubricosa Dearn. and Barth., which, as shown later, is the same fungus as *Mastigosporium calvum*, was collected on *Calamagrostis scabra* Presl from Glacier National Park, Mont., in August 1915 (*4*). The host, which is now generally known as *C. canadensis* var. *scabra* (Presl) Hitchc., is a plant of subalpine and alpine areas in the northern United States and Canada. It is interesting to note that Frank (*5*) found a muticate form of *M. album* on *Alopecurus pratensis* on the highest parts of the Erzgebirge of Saxony, Germany. In commenting on this, Lindau (*10*) wondered whether the muticate spores were due to the altitude or to a species difference. It is noted in Oregon that most of the collections of *M. calvum* have been made at elevations between 0 and 400 feet above sea level. Whether or not *M. calvum* occurs on alpine and subalpine grasses in Oregon has not been determined.

SYMPTOMS CAUSED BY MASTIGOSPORIUM CALVUM

SYMPTOMS ON DACTYLIS GLOMERATA

The disease caused by *Mastigosporium calvum* on *Dactylis glomerata* is first visible as small, dark, purple-brown flecks on the leaves. Some of these enlarge, becoming elliptical in outline, with ashy-gray to fawn-color centers. The lesions measure 1 to 8 mm in length and are somewhat restricted by the parallel veins of the leaf (fig. 3). Where the fungus is able to make rapid headway or where secondary infection from spores occurs the lesions may be larger, somewhat mottled, and with less definite borders. These larger lesions show various shades of gray, ashy, or light-fawn color, with more or less definite purple, red, or ocher borders. Any or all leaves of a plant may be attacked, as the fungus is an active parasite and may practically defoliate the orchard grass plants.

The presence of long, dead striae, with black dots of conidiophore tufts, is indicative of the presence of the streak disease caused by *Scolecotrichum graminis* Fckl., which is frequently associated with the mastigosporium disease in Oregon. The symptoms of the streak disease, which are well described by Horsfall (*7*), are sometimes confusable with those of the mastigosporium leaf spot. The purple

flecking mentioned for the latter is sometimes duplicated in early stages of the streak disease. Usually, however, *Mastigosporium calvum* causes a more abundant development of pigments than does *S. graminis*, and, furthermore, the latter produces longer lesions, usually with rather conspicuous black tufts of conidiophores.

SYMPTOMS ON AGROSTIS SPECIES

The symptoms produced by *Mastigosporium calvum* on *Agrostis* spp. differ from those on orchard grass in the tendency to form broader, elliptical, light-brown lesions with light-fawn centers. The lesions are often of an eyespot or frogeye type on account of a broad red or red and yellow margin. On redtop the lesions were sufficiently numerous to destroy most of the leaves of plants in certain fields near Astoria in 1936 and1937. On Astoria colonial bent the spots averaged from one to six per leaf with a tendency to develop at the tips or where moisture collected at the base of the leaf blades. In 1937 colonial bent was heavily infected during April and May in Clatsop and Tillamook Counties.

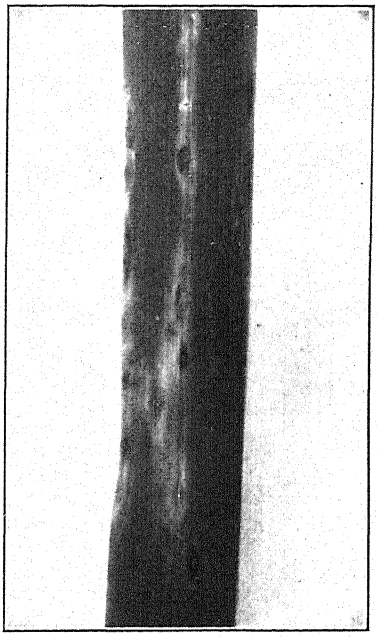


FIGURE 3.—Leaves of *Dactylis glomerata* attacked by *Mastigosporium calvum*, Corvallis, Oreg. × 2.

SYMPTOMS ON CALAMAGROSTIS SPECIES

On species of *Calamagrostis*, *Mastigosporium calvum* produces small brown flecks, 1 to 3 mm in diameter, with very small gray centers. The entire lesion is sometimes surrounded by larger areas of fawn- or pale buff-colored tissue. Specimens from Montana showed extensively coalesced, mottled lesions.

SYMPTOMS CAUSED BY MASTIGOSPORIUM ALBUM IN EUROPE

While field comparisons have not been made, it is doubtful whether *Mastigosporium calvum* and *M. album* can be readily distinguished in the field except possibly by hosts. *M. album* is the more common, in exsiccati at least, on *Alopecurus pratensis* and *Deschampsia caespitosa* (L.) Beauv., while *M. calvum* prefers *Dactylis glomerata*, *Agrostis* spp., and *Calamagrostis* spp. Lindau (*10*), however, lists a number of hosts other than these for *M. album*. Since he has not distinguished entirely between the muticate and nonmuticate collections, and since these collections are not available to the writer, it is not possible to give a more extensive host range. Lindau lists, in addition to the ones cited, *Brachypodium sylvaticum* (Huds.) Beauv., *Molinia caerulea* (L.) Moench, *Avena elatior* L., *Briza media* L., *Glyceria fluitans* (L.) R. Br., *Holcus lanatus* L., *H. mollis* L., *Phleum pratense* L., *Poa trivialis* L., and *Trisetum flavescens* (L.) Beauv.

The following notes on symptoms are based on an examination of exsiccati and a study of the brief reports in literature.

SYMPTOMS ON ALOPECURUS PRATENSIS

On *Alopecurus pratensis* there usually are numerous small elliptical purple-brown to dark-brown flecks which later enlarge and develop white to ashy-gray centers. The coloration on *A. pratensis* is deeper than for most collections of *Mastigosporium calvum* on *Dactylis glomerata*, but this probably is attributable as much to host reaction as to differences in the two fungi.

SYMPTOMS ON DESCHAMPSIA CAESPITOSA

On *Deschampsia caespitosa* (*Aira caespitosa* L.) the symptoms are very similar to those on *Alopecurus pratensis* except that the spots on the former grass seem to be slightly less deeply pigmented than those on the latter, as indicated by the specimens in De Thümen, Myc. Univ., 1872.

PURE-CULTURE STUDIES

MASTIGOSPORIUM CALVUM FROM DACTYLIS GLOMERATA

Pure cultures of *Mastigosporium calvum* were obtained from spores washed from leaves of orchard grass collected at Corvallis, Oreg. The fungus, which grows relatively slowly on potato-dextrose agar, produces a leathery, somewhat folded and mounded colony which becomes pebbly or roughened by the formation of small, partially embedded, stromatic or sclerotic mycelia that resemble pycnidia. Brief descriptions of the development of the fungus during the first month after transfer of diseased host tissue to four kinds of nutrient media are given in table 2.

TABLE 2.—*Development of mycelia of Mastigosporium calvum on four kinds of nutrient agar at 35° to 40° F. in total darkness*

Agar culture medium	Color of—		Description of colony
	Substratum	Aerial mycelia	
Difco corn decoction	Yellow	Straw, later yellow	Stellate-margined, leathery wrinkled colonies, partially submerged.
Mix's synthetic	White	White	Flat, thin, spreading colonies.
Difco potato dextrose	Pale umber	Tan to fawn	Leathery, pebbly mounded, wrinkled colonies.
Difco prune	Pale cocoa	Pale cocoa to fawn	Less mounding, margins more stellate.

Normal-appearing conidia were produced in moderate quantities on the surface of the colonies on potato-dextrose agar after incubation for several weeks in the ice box at 35° to 40° F. After growing several months at this temperature the colonies finally became orange brown and the sclerotia increased in number, frequently forming rings in the substratum around the original point of transfer.

MASTIGOSPORIUM CALVUM FROM AGROSTIS SPECIES

Pure cultures of *Mastigosporium calvum* were isolated from *Agrostis alba*, *A. canina*, and *A. tenuis* collected at Astoria, Oreg.

MASTIGOSPORIUM CALVUM FROM ALOPECURUS

A culture of *Mastigosporium album* from *Alopecurus pratensis*, kindly forwarded by Kathleen Sampson from Aberystwyth, Wales,

had the same general yellow-brown color of *M. calvum* but produced masses of conidia in glistening orange-brown mounds on the surface of the colony. After transfer to potato-dextrose agar the sporulation continued but was at first putty-colored, later darker. The conidia were typically navicular, three- to five-septate, and bore the characteristic appendages at the apex. The fact that this fungus continues to produce typical conidia in culture that do not revert to the muticate type is additional strong evidence that the muticate and ciliated species are distinct.

DILOPHOSPORA ALOPECURI FROM HOLCUS LANATUS

Since Rainio (*11*) has strongly contended that *Mastigosporium album* is an early stage of *Dilophospora alopecuri* (Fries) Fries, the writer isolated cultures of the latter fungus from *Holcus lanatus* from Oregon. This fungus grew very slowly, and germinating spores required nearly 8 weeks to produce macroscopically visible colonies when incubated at 38° F. After 6 months the colony covered the surface of test-tube slants with a mounded, felty, olive and gray growth, which was covered with guttulae. These cultures, therefore, differed distinctly from those of *M. calvum*.

INOCULATION STUDIES

Water suspensions of viable conidia of *Mastigosporium calvum* taken from naturally infected plants in the field were sprayed with an atomizer on pot-grown, healthy grasses in the greenhouse at Corvallis. The inoculated plants, together with adequate checks, were incubated in a moist chamber for 1 week. The first series, conducted in January 1934, and a second series, conducted the following month, produced a light infection on orchard grass only. Another more extensive series of inoculations was conducted in May 1937 with *M. album* from Wales and with spore washings of *M. calvum*, both from *Dactylis glomerata* and *Agrostis alba*. The results are shown in table 3.

TABLE 3.—*Results of inoculating grasses in the greenhouse with spore suspensions of Mastigosporium album and M. calvum*

Host	Inoculated with *M. album* from *Alopecurus pratensis*, May 1, 1937		Inoculated with *M. calvum* from—							
			Dactylis glomerata						*Agrostis alba*, May 1937	
			January 1934		February 1934		May 1937			
	Leaves infected	Leaves not infected	Leaves infected	Leaves not infected	Leaves infected	Leaves not infected	Leaves infected	Leaves not infected	Leaves infected	Leaves not infected
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Agrostis palustris Huds.	0	190	-------	-------	-------	-------	0	400	10	265
A. tenuis Sibth.	0	175	-------	-------	-------	-------	0	215	78	65
Alopecurus pratensis L.	38	46	-------	-------	-------	-------	0	80	0	240
Avena sativa L.	-------	-------	-------	-------	0	48	0	90	0	98
Brachypodium pinnatum (L.) Beauv.	6	30	-------	-------	-------	-------	-------	-------	-------	-------
Bromus rigidus Roth.	0	15	-------	-------	-------	-------	-------	-------	0	120
Dactylis glomerata L.	0	205	5	206	39	180	31	0	10	290
Deschampsia caespitosa (L.) Beauv.	16	227	-------	-------	-------	-------	0	165	0	190
Festuca elatior L.	0	150	-------	-------	-------	-------	-------	-------	-------	-------
F. rubra L.	-------	-------	-------	-------	0	60	-------	-------	-------	-------
Holcus lanatus L.	0	51	-------	-------	0	84	0	88	0	155
Lolium perenne L.	-------	-------	0	63	0	79	0	100	0	180
Phleum pratense L.	0	40	-------	-------	-------	-------	-------	-------	-------	-------
Poa pratensis L.	0	75	0	52	0	75	0	85	-------	-------
Triticum aestivum L.	0	36	-------	-------	0	45	0	25	-------	-------

It will be noted that *Mastigosporium album* from *Alopecurus pratensis* attacked that host, *Brachypodium pinnatum*, and *Deschampsia caespitosa*; *M. calvum* from *Dactylis glomerata* attacked only that host, and this fungus from *Agrostis alba* attacked *A. palustris*, *A. tenuis*, and *D. glomerata*.

While further inoculations are needed, the present results indicate (1) that *Mastigosporium album* and *M. calvum* differ in host range as well as in morphology, and (2) that there are distinct physiologic races in *M. calvum*.

MORPHOLOGY OF MASTIGOSPORIUM CALVUM

In pure cultures of *Mastigosporium calvum* the conidia are produced on the tips of hyphae (fig. 4). The distal portions of the conidia, following the constriction at the base of the conidiophore, develop faster than the basal portions. In the young conidium there are two nuclei which divide and form four cells in the mature spore. The four-celled condition is typical and is remarkably uniform. Sometimes spores with only two cross walls are found, but these are immature spores with the basal portions incompletely developed.

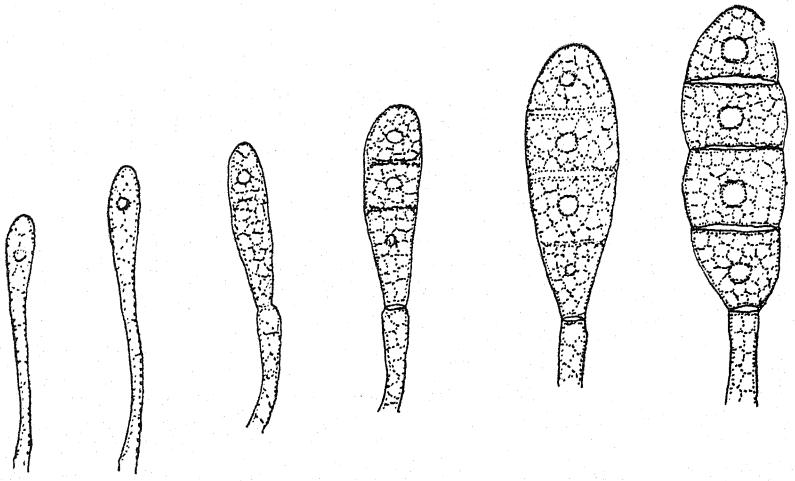


FIGURE 4.—Stages in the development of conidia of *Mastigosporium calvum* in pure culture on potato-dextrose agar at 40° F. × 1,075. (Diagramed from photomicrographs.)

On the host the development of the conidia (fig. 5) is approximately the same as on media. The spores are borne on short conidiophores which emerge directly from the leaf tissue, usually between the epidermal cells or through stomata. Stromatic development is moderate to slight, the fungus developing between the epidermal cells or in the cells.

Conidia usually are produced in relatively moderate numbers, and if the lesions are small and immature, few if any spores develop. On some of the bentgrasses larger numbers of spores were found, but the conidia do not occur in masses as in *Fusarium* or *Septogloeum*. The conidia, in addition, are somewhat brittle, and one or more cells may collapse, particularly under desiccation. As indicated from germina-

tion trials, the conidia apparently are fitted for quick germination and are relatively short-lived. They germinate from the apical end, producing one or two stout germ tubes which soon branch repeatedly.

The appendages or cilia that occur on conidia of the type material of *Mastigosporium album*, and which are characteristic of the genus on a majority of the reported collections from Europe, are apical or subapical, hyaline, tentaclelike protuberances (fig. 1, *J–O*). They resemble, except that they are considerably narrower, the mycelia from germinating conidia of *M. calvum* seen on potato-dextrose agar at Corvallis. Since, however, their position, shape, and length are fairly constant, they appear to be normal outgrowths formed as the result of some physiologic reaction which is not understood at present. They are believed to represent further development of the apical portions of the conidia and probably are morphologically akin to arrested germ tubes. Saccardo was uncertain about their nature, as indicated from his remarks (*14*).

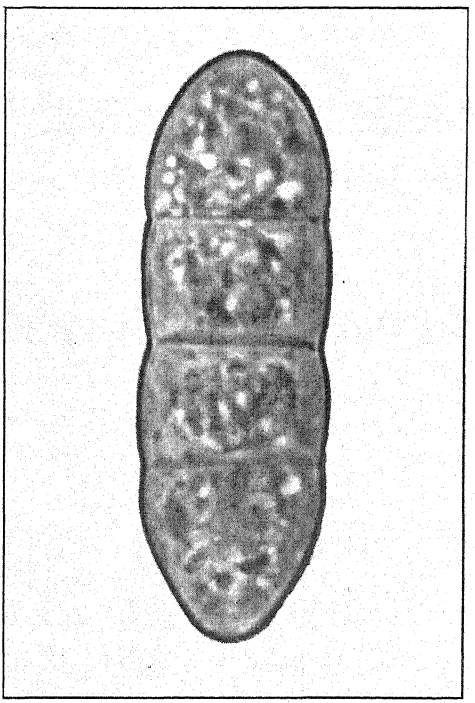


FIGURE 5.—Photomicrograph of conidium of *Mastigosporium calvum* on *Dactylis glomerata*, Corvallis, Oreg. × 2,000.

TAXONOMY

The appendaged *Mastigosporium album* and the non-appendaged *M. calvum* are distinct species if the present criteria for segregating species of fungi are logical. The two fungi are distinct because—

(1) The spores of *M. album* often have four cross walls, sometimes five; those of *M. calvum* have never more than three.

(2) The spores of *M. album* have appendages; those of *M. calvum* have not.

(3) The spores of *M. album* usually are longer and sometimes narrower than those of *M. calvum*.

(4) The two fungi maintain their respective characteristics in artificial culture on nutrient media.

(5) The host range of each fungus is fairly distinct.

A study of fresh material sent from Wales by Kathleen Sampson shows that the two fungi are, however, closely related. Both have coarse mycelia, which produce short, thick conidiophores. In their early stages the fungi appear almost identical, and until the tip of *Mastigosporium album* starts to elongate they appear to be the same species. *M. album*, however, grows from the apical end, producing the awl-shaped cell which frequently develops one or two cross walls in addition to the usual three cross walls in the main body of the spore. The apical cell then continues to elongate into the characteristic

appendage, which may become once, or more, forked. Additional appendages are sometimes formed from the side of the apical cell or cells. There is no question in the writer's mind that both fungi belong in the same genus and have arisen apparently from the same original species. The writer has considered the hypothesis that on certain hosts *M. album* produces appendages and on others not, and that this is a reaction to the host. From comparison with pure cultures this hypothesis appears unlikely. Therefore he proposes making two species in the genus *Mastigosporium*.

The genus *Mastigosporium* needs some revision to conform to the fungi included. It belongs in subsection Micromereae of section Hyalophragmeae of the Moniliaceae. This group includes among others *Fusoma* Corda and *Amastigosporium* Bond.-Mont. *Fusoma* has a very vague status as a fungus, with fusiform septate spores borne on scanty mycelia. Neither of the species of *Mastigosporium* appears to belong in *Fusoma*, although *F. rubricosa*, which is a synonym of *M. calvum*, has been described (*4*).

Amastigosporium graminicolum Bond.-Mont. (*1*) is the same as *M. calvum*. *Amastigis graminicola* Bond.-Mont. appears to be a simplified spelling of *Amastigosporium* attributed to Clements in Clements and Shear (*2*).

Mastigosporium is emended as follows:

Mastigosporium Riess (Emended).

Syn.: *Monothecium* Lib. in herb. and *Amastigosporium* (*Amastigis*) Bond.-Mont.

Mycelia in plants, epidermal and subepidermal, endophytic or somewhat ectophytic, septate, coarse, branched, hyaline; conidiophores, brief single or in small groups, short stipitate, continuous; conidia hyaline elliptical, large, navicular to subulate, formed by expansion of the hyphal tips, three-to-five septate, apical cells of conidia rounded and muticate, or awl-shaped and appendaged with simple or forked usually aseptate filiform appendages.

Included species, *Mastigosporium album* Riess (appendaged) and *M. calvum* (Ellis and Davis) comb. nov. (nonappendaged). Excluded, *M. lupini* (Sor.) Cav.

Mastigosporium lupini is excluded solely because of its pigmentation. Its manner of producing spores is remarkably like that of *M. album* and *M. calvum*.

Because it is virtually impossible to locate *Mastigosporium calvum* by use of present keys, it is suggested that future analytical keys to genera of fungi take cognizance of both the appendaged and nonappendaged species of *Mastigosporium*.

The data on spore size for available collections are presented in table 4. Apparently the two species are the same size until the elongation of the tip of *Mastigosporium album* starts. Most collections of the latter therefore average longer in mature spores than *M. calvum*.

In comparing the relatively fresh material from Wales with older exsiccati it appears that *Mastigosporium album* shrinks considerably after prolonged desiccation. The difference in width of the spores of the two species does not appear to be as great as indicated in table 4.

Mastigosporium calvum occurs on *Dactylis glomerata, Calamagrostis* spp., and *Agrostis* spp. Jaap (*3*) and Frank (*5*) report muticate spores of a *Mastigosporium* on *Alopecurus pratensis* which may be *M. calvum*. The fungus has not been found on *A. pratensis* in Oregon, although *M. calvum* occurs on *Dactylis* within short distances of small plots of *A. pratensis* on the experiment station farms at Corvallis and Astoria (table 1).

TABLE 4.—*Host, exsiccati number, ciliation (+ or —), location, conidial size, etc., of Mastigosporium album and M. calvum on grasses in Europe and North America*

Fungus	Host	Exsiccati, authority, or literature citation	Ciliation, verified by examination of exsiccati	Geographic location	Size of conidia	Appendages
	Alopecurus pratensis L.	Riess (*6, p. 56*)	(1)		55 × 12 μ	+
	Deschampsia caespitosa (L.) Beauv.	De Thümen, Myc. Univ., 1872	+	Belgium	45–55 × 9–12	+
	A. pratensis L.	Sydow, Myc. Ger., 542	+	Germany	43–49 × 8–13	+
	do	Buch. et Bond. Fungi Ross. Exs., Ser. A, 197.	+	Union of Soviet Socialist Republics.	50–51 × 11–13	+
M. album	do	Linhart, Fungi Hungar., 96	+	Hungary	50–55 × 13	+
	do	Krieger, Fungi Sax., 790 A and B	+	Germany	43–50 × 9–14	+
	do	Kabát et Bubák, Fungi Imp. Exs., 386	+	Bohemia	38–51 × 10–11	+
	do	Schroeter	—	Germany	48–55 × 11–13	+
	do	K. Sampson (Oreg. 8248)	+	Wales	31–53 × 12–16	+
Range of conidial size					31–55 × 8–16	
	Agrostis alba L.	Oregon 10586	+	Oregon	32–51 × 12–16	—
	do	Oregon 8268	+	do	39–47 × 13–17	—
	A. palustris Huds.	Oregon 10308	+	do	29–38 × 10–15	—
	do	Oregon 8246	+	do	40–46 × 14–16	—
	A. tenuis Sibth.	Oregon 10585	+	do	35–45 × 11–16	—
	do	Oregon 10584	+	do	36–38 × 11–13	—
	A. verticillata Vill.	Oregon 8262	+	do	38–45 × 13–16	—
	Calamagrostis canadensis (Michx.) Beauv.	Herb., J. J. Davis	+	Wisconsin	40–45 × 11.5–12	—
	do	Univ. Toronto, Crypt. Herb., 1292	+	Ontario	33–40 × 11–14	—
	C. canadensis var. *scabra* (Presl) Hitchc.	Fungi Columb., 5019	+	Montana	37–44 × 10.7–14	—
M. calvum	*C. epigeios* (L.) Roth	Sydow, Myc. Ger., 640	+	Germany	40–50 × 14–16	—
	C. halleriana	Krieger, Fungi Sax., 791	+	do	35–42 × 11–12	—
	Dactylis glomerata L.	Saccardo (*14*)	—	France	30–32 × 11	—
	do	Wakefield (*16, p. 253*)	—	England	32–38 × 10–12	—
	do	Oregon 10583	+	Oregon	40–45 × 13–15	—
	do	Oregon 10044	+	do	30–41 × 12–14	—
	do	Oregon 10011	+	do	34–38 × 10–12	—
	do	Oregon 10094	+	do	30–39 × 11–13	—
	do	Oregon 10205	+	do	31–37 × 10–12	—
	do	Oregon 10322	+	do	34–37 × 9–12	—
	do	Krieger, Schol. Pilze, 192	+	Germany	32–36 × 11–12	—
	do	Krieger, Fungi Sax., 792	+	do	35–40 × 12–14	—
	do	Bondarzeva-Monteverde (*1*)	—	Union of Soviet Socialist Republics.	40–60 × 14–16	—
Range of conidial size					29–60 × 9–17	

[1] Illustrations showed ciliated spores.

The earliest, although brief, description of *Mastigosporium calvum* appears to be that of *M. album* var. *calvum* Ellis and Davis (*3*). The combination *M. calvum* (Ellis and Davis) comb. nov. is proposed and the following synonymy listed:

Mastigosporium calvum (Ellis and Davis) comb. nov.

Syn.: *M. album* var. *calvum* Ellis and Davis, 1903.
M. album var. *muticum* Sacc., 1911.
Fusoma rubricosa Dearn. and Barth., 1917.
Amastigosporium graminicola Bond.-Mont., 1921.
Amastigis graminicola Bond.-Mont. (Clements), 1931.

Excluded, *M. album* var. *athrix* Eriks.

Wollenweber proposed *Bactridium triseptatum* (Sacc.) Wr. in his illustrated card index (*17*). His illustration 457 is based on Jaap's Fungi Rel. Exs. 494 and is typical for *Mastigosporium calvum* on *Calamagrostis*. His illustration 458, however, shows a typical species of *Bactridium* (*B. gymnosporangii* (Jaap) Wr.) that has distinct, somewhat elongated conidiophores arising from a small but definite sporodochial pad. *M. calvum* can scarcely be placed under *Bactridium*. To have placed this fungus under the combination *B. triseptatum*, Wollenweber must have seen the type of *Fusoma triseptatum* Sacc. If this material were *M. calvum*, Saccardo may have fallen into a trap, because his description and his illustration (*12*) and those of the apparently identical species *F. biseptatum* Sacc. (*13, p. 69*) are apparently those of a very much smaller-spored fungus referable to *Septogloeum oxysporum* Bomm., Rouss., and Sacc. Saccardo names his fungus *F. triseptatum* and then lists it as two-septate, while *M. calvum* is triseptate. His later description of *F. biseptatum* is singularly like his earlier one of *F. triseptatum*. It is possible that both *M. calvum* and *S. oxysporum* occur in the type of *F. triseptatum*. However, the published description is the final criterion; and *M. calvum* cannot possibly be referred to *F. triseptatum*.

Mastigosporium album var. *athrix* Eriks. is a *Septogloeum* and should be known as *S. athrix* (Eriks.) comb. nov. (fig. 1, *P*). It is highly probable that it may be the same as *S. oxysporum*.

The writer has found no evidence that *Mastigosporium calvum* is connected with *Dilophospora alopecuri*. In Oregon material of *D. alopecuri* on *Holcus lanatus*, it certainly does not appear to be connected with *M. album*. *M. album* has not been found in Oregon, and Miss Sampson's pure culture of it appears to be entirely different from a pure culture of the race of *D. alopecuri* on *H. lanatus*.

SUMMARY

Mastigosporium calvum (Ellis and Davis) comb. nov. causes a purple flecking and a leaf spot on *Dactylis glomerata* and an eyespot on the leaves of several species of *Agrostis* in northwestern Oregon. The fungus is a destructive parasite on these grasses during mild, rainy weather in winter and spring. It occurs on *Calamagrostis* spp. in Wisconsin. It also occurs on these grasses in Europe. It has been considered a variety of *M. album* Riess, which differs from it in having hyaline apical appendages, whereas *M. calvum* is muticate. *M. album*, which is common on *Alopecurus pratensis* and *Deschampsia caespitosa* in Europe, has not been reported from North America.

The genus *Mastigosporium* is emended to include elliptical, three- to five-septate, hyaline conidia borne on short, stipitate conidiophores. The apex of the conidia may have appendages (*M. album*) or may not have appendages (*M. calvum*).

No evidence was obtained that *Mastigosporium album* Riess is related to *Dilophospora alopecuri* (Fries) Fries.

Mastigosporium album var. *athrix* Eriks. is assigned to *Septogloeum athrix* (Eriks.) comb. nov., and its possible relation to *S. oxysporum* is mentioned.

LITERATURE CITED

(1) Bondarzeva-Monteverde, V.
1921. [On the microflora of lower Orlovski. two new parasitic fungi.] Materialy po Mikologicheskomu Obsledovaniiu Rossii 5, No. 1, 4 pp. [In Russian. Description of fungi in Latin.]

(2) Clements, Frederic E., and Shear, Cornelius L.
1931. the genera of fungi. 496 pp., illus. New York.

(3) Davis, J. J.
1903. third supplementary list of parasitic fungi of wisconsin. Wis. Acad. Sci., Arts, and Letters, Trans. 14: [83]–106.

(4) Dearness, John.
1917. new or noteworthy north american fungi. Mycologia 9: 345–364.

(5) Frank, A. B.
1896. i. mastigosporium riess. *In* his Krankheiten der Pflanzen, Aufl. 2, v. 2, pp. 356–357. Breslau.

(6) Fresenius, Georg.
1850–63. beiträge zur mykologie. 111 pp., illus. Frankfurt-a.-M.

(7) Horsfall, James G.
1930. a study of meadow-crop diseases in new york. N. Y. (Cornell) Agr. Expt. Sta. Mem. 130, 139 pp., illus.

(8) Jaap, Otto.
1914. ein kleiner beitrag zur pilzflora von thüringen. Ann. Mycol. 12: 423–437.

(9) Jorstad, I.
1924. beretning om plantesygdommer i land og hagebruket i 1922–23. iv. landbruksvekster og grønnsaker. 38 pp. Christiania. [Abstract in Rev. Appl. Mycol. 4: 16–18. 1925.]

(10) Lindau, G.
1907. fungi imperfecti: hyphomycetes. (erste hälfte). *In* Rabenhorst L., Kryptogamen-flora von Deutschland, Oesterreich und der Schweiz. Aufl. 2, Bd. 1, Abt. 8. Leipzig.

(11) Rainio, A. J.
1936. über die dilophospora-krankheit von phleum pratense l. und alopecurus pratensis l. Valtion Maatalouskoet. Julkaisu. 87, 32 pp., illus. [In German. Selostus in Finnish, pp. [30]–32.]

(12) Saccardo, P. A.
1892. fusoma triseptatum sp. nov. *In* his Sylloge Fungorum . . ., v. 10, pp. 566–567. Patavii.

(13) ———
1893. fungilli novi europaei et asiatici. Grevillea 21 (99): [65]–69.

(14) ———
1911. notae mycologicae. Ann. Mycol. 9: [249]–257.

(15) Stapledon, R. G., Williams, R. D., Sampson, Kathleen, and Jenkin, T. J.
1922. preliminary investigations with herbage plants. Welsh Plant Breeding Sta. Aberystwyth, Bull. Ser. H, No. 1, 97 pp.

(16) Wakefield, E. M.
1918. new and rare british fungi. Roy. Bot. Gard. Kew, Bull. Misc. Inform. 1918 (6): 229–233.

(17) Wollenweber, H. W.
1917. fusaria autographice delineata . . . 56 pp. Berlin. (Extract from Ann. Mycol. v. 15, Nos. 1–2, 1917.)

PRELIMINARY BIOCHEMICAL STUDIES ON EFFECTS OF CERTAIN ENVIRONMENTAL FACTORS ON DEVELOPMENT AND COMPOSITION OF THE PEANUT[1]

By SAMUEL L. JODIDI[2]

Physiologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The peanut (*Arachis hypogaea* L.) is one of the important crops grown in the southern part of the United States. About 2,000,000 acres of land are devoted to the crop in this country, producing about a million tons of unshelled[3] peanuts annually (*16, p. 572*).[4] In times of normal prices, the annual crop has a farm value of about $25,000,000.

The plant is of relatively great importance as a cash crop grown for the manufacture of oil, peanut butter, confectionery, salted peanuts, and stock feeds, and it is extensively grown for stock feeding on the farm (*2, p. 26; 17, p. 7*). Despite its importance, the peanut has had comparatively little careful study from the biochemical standpoint, except with reference to the properties of its oil and certain proteins. There are numerous growth and production problems that have important relationships not only to yields of unshelled peanuts per acre but also to filling of pods, quality of kernels, and adaptation of different types and varieties to specific regions or other conditions of culture.

Prior to the completion of the original draft of this paper the author found no literature on the physiological and biochemical behavior of the peanut with reference to the normal or desirable and abnormal or undesirable results often obtained under field conditions. Since then a report of studies of nutritional levels in the peanut plant by Moore (*11*) has appeared. Moore, working at Chicago, Ill., grew peanut plants in the greenhouse, in pots of quartz sand, with a series of nutrient solutions designed to produce wide differences in vegetative and reproductive behavior. Different fractions of the plants and fruits were analyzed for various forms of carbohydrate and nitrogenous substances. In general, the nutritional conditions resulted in unfruitfulness, offering no basis for comparison with the present studies. However, one comparison of high versus very low nitrogen supply showed for both shells and seeds a lower total sugar, lower total carbohydrate, and higher total nitrogen content. Data on plant size and yields and character of fruits were lacking, precluding comparisons with results of the present paper.

[1] Received for publication November 29, 1937; issued August 1938.

[2] The peanut samples were obtained from certain variety and fertilizer plots of the Virginia Agricultural Experiment Substation at Holland, Va., through the courtesy of that institution. Samples 5 and 6 were obtained from the plots of a preliminary spraying investigation being conducted cooperatively by the Virginia Agricultural Experiment Station and the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, in which workers in the Division of Fruit and Vegetable Crops and Diseases are cooperating informally. The author acknowledges the kindness of these workers in making this material available.

[3] The word "unshelled" refers to peanuts in the shell throughout this paper.

[4] Italic numbers in parentheses refer to Literature Cited, p. 310.

The large-seeded Virginia type of peanut is apparently adapted to a very restricted range, which comprises an area about 50 to 60 miles in radius in southeastern Virginia and northeastern North Carolina and a smaller district in central Tennessee. The Spanish type of peanut, on the other hand, is generally well established throughout the southeastern quarter of the United States. The reasons for these very marked differences in adaptability are not at all understood.

A common cause of low yields of cleaned unshelled peanuts per acre and of low ratios of shelled to unshelled peanuts is the formation of many pods that contain poorly developed or quite undeveloped seeds. Varieties are known to differ in the extent to which they show this trouble, and the same variety may show widely differing degrees of it in various fields, years, or regions. It is not known to what extent light, temperature, moisture, mineral nutrients, soil texture, or cultural methods, either alone or together in various combinations, affect the development of plants and fruits in the regions where the crop is commonly grown.

The average yields and the efficiency of crop production in general are so far below the apparent possibilities that there is a pressing need for exhaustive fundamental studies of the plant in all stages of development and from numerous points of view—anatomical, morphological, and genetic, as well as biochemical and biophysical.

Certain cultural studies and seed-stock-improvement work on peanuts have been in progress for several years in the United States Department of Agriculture and have yielded results of definite value, and recently the Division of Fruit and Vegetable Crops and Diseases of the Bureau of Plant Industry has initiated studies of a more technical nature designed to solve some of these more fundamental problems.

The present paper reports the results of chemical examination of kernels and shells from six preliminary lots of peanut plants, which varied markedly in yield and appearance and which grew on soils of different texture or received different fertilizer or other chemical treatment. The object of this preliminary examination was to determine whether markedly different development and yield responses were accompanied by marked and easily determinable differences in physiological condition as revealed by estimation of certain chemical constituents of the fruits. These studies will be extended to other portions of the plant, and samples will be studied from a wide variety of natural field and controlled experimental conditions.

MATERIAL AND METHODS

EXPERIMENTAL PLOTS

The data concerning the fertilizer treatments of the experimental plots at Holland, Va., the peanut strains grown, etc., can readily be seen by reference to table 1, which shows that the yields varied markedly between varieties and treatments, but the yields per plant shown cannot be taken as typical of the response of entire plots receiving the respective treatments. In some instances the samples were selected from spots in the large plots in order to obtain varying degrees of plant size and vigor, regardless of the behavior of the plot as a whole, or to examine varietal differences. Nevertheless, the treatments of the various plots are presented as being of some interest.

The apparent influence of bordeaux mixture treatment on the yield is interesting. The Jumbo Virginia Runner of plot 5, which received, in addition to 1 ton of lime, three bordeaux mixture applications, gave 173.1 g of peanuts per plant as compared with 94.5 g for plot 6, which received the same amount of lime but no bordeaux mixture treatment. It seems reasonable to attribute the difference in part to the stimulating effect of copper, as shown by the considerably more vigorous growth and deeper green color of the plants in plot 5 as compared with those in plot 6.

TABLE 1.—*Type of soil, treatment, peanut varieties grown, and yields obtained from the various plots, 1934*

Plot No.	Type of soil	Treatment [1]	Variety	Plants taken for analysis	Average weight of unshelled peanuts per plant	Mean weight of peanuts per pod	Relative weight of shelled peanuts
				Number	*Grams*	*Grams*	*Percent*
1	Onslow fine sandy loam of lighter texture.	300 pounds of gypsum applied Aug. 1, 1933.	Small Virginia Runner.	20	105.0	0.93	66.0
2	----do----	300 pounds of gypsum applied Aug. 1, 1933, followed by 40 pounds of K_2O from kainite at planting.	Jumbo Virginia Runner.	50	40.8	1.48	67.0
3	Onslow fine sandy loam. Low spot of heavy texture.	300 pounds of gypsum applied Aug. 1, 1933, followed by 143 pounds of cottonseed meal at planting.	----do----	40	58.6	1.55	67.0
4	Onslow fine sandy loam of lighter texture.	300 pounds of gypsum applied Aug. 1, 1933 (check).	----do----	34	65.6	1.18	63.7
5	Onslow fine sandy loam of heavier texture.	1 ton of ground oystershell lime applied Apr. 1, 1934, followed by 3 bordeaux mixture applications (4–4–50) on July 15, Aug. 1 and 15, 1934.	----do----	13	173.1	1.38	65.5
6	----do----	1 ton of ground oystershell lime applied Apr. 1, 1934, but not treated with bordeaux mixture (check).	----do----	20	94.5	1.38	67.5

[1] Peanuts designated in pounds represent rates per acre.

PRESERVATION OF SAMPLES

The peanuts were picked in the field, put in paper bags, and within 6 to 7 hours were placed on a screen, shaken to free them from adhering soil, weighed, and transferred immediately to a forced-draft drying oven, where a temperature of 65° to 70° C. was maintained for about 20 hours. The moisture estimations of the fresh peanuts were not considered reliable enough to be recorded, because the plants had been pulled from the soil and exposed to an undetermined degree of drying for some hours before samples were taken. After the preliminary drying, the carefully cleaned peanuts were shelled and the kernels and shells ground separately in an electrically driven mill. The shells were passed through a 40-mesh sieve, but the kernels could not be so sifted because they had been ground somewhat coarsely to prevent any oil from being squeezed out. Since ground kernels at room temperature are known to become rancid and perhaps to undergo other changes with time, the samples were kept at about 2° until analyses were completed. Since the minced materials were placed

in tightly covered jars immediately after being ground and before their moisture content had reached equilibrium with that of the surrounding air, accurate moisture determinations were made in duplicate at the beginning and toward the conclusion of the investigation to ascertain whether the moisture content had changed during the time of making the analyses and, if so, to what extent.

Table 2 shows that when the dry-matter estimations made in October 1934 are compared with those of June 1935 the greatest difference is 0.32 percent in the case of the kernels (plot 1) and 0.55 percent for the shells (plot 2). It therefore appeared perfectly safe to calculate the results of the estimations of ash, oil, sugars, protein, etc., on the basis of dry matter found in October 1934. It is of interest to note that, after the unshelled peanuts had been dried at 65° to 70° C. for 20 hours, the separated kernels and shells from the various plots showed very similar percentages of dry matter, respectively, and with but one exception differed by only a few tenths of 1 percent.

TABLE 2.—*Percentage of moisture*[1] *in peanut samples preserved by drying at 65°–70° C. for 20 hours*

Plot No.	Moisture in—				Plot No.	Moisture in—			
	Kernels		Shells			Kernels		Shells	
	Immediately after preservation drying at 65°–70° C. in October 1934	After storing for 8 months at 2° C. in closed jars	Immediately after preservation drying at 65°–70° C. in October 1934	After storing for 8 months at 2° C. in closed jars		Immediately after preservation drying at 65°–70° C. in October 1934	After storing for 8 months at 2° C. in closed jars	Immediately after preservation drying at 65°–70° C. in October 1934	After storing for 8 months at 2° C. in closed jars
	Percent	*Percent*	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*	*Percent*
1	3.33	3.65	4.06	3.54	4	3.25	3.23	3.70	3.53
2	2.95	3.22	4.26	3.71	5	3.27	3.00	3.79	3.37
3	3.10	3.34	3.56	3.23	6	3.48	3.52	4.42	4.39

[1] Moisture was determined at 105° C. just after initial drying and after 8 months' storage in closed containers at 2° C.

CHEMICAL ANALYSES

ASH CONTENT

In order to obtain uniform, strictly comparable ash determinations, duplicate portions from the six samples were incinerated simultaneously in an electric muffle furnace at a red heat below the fusion point of the ash. In each case a loose, homogeneous ash was obtained. The average percentages found are presented in table 3.

As will be seen, the kernels from the several plots showed considerable differences in the percentages of ash, which ranged from 2.22 for plot 5, with maximum growth and yield, to 2.56 for plot 2, with least growth and yield. For the shells, the lowest ash content was 2.73 percent for plot 3 (heavy soil high in organic matter) and the highest 4.04 percent again for plot 2. The variety of peanut, as well as the type of soil and the fertilizers applied, may have been a factor. The ash content of the shells was considerably larger than that of the corresponding kernels from each of the plots, the average ash content of the shells for all six plots being about 38 percent higher than that of the kernels.

TABLE 3.—*Percentage of total ash in peanut kernels and shells, determined on moisture-free basis*

Plot No.	Variety	Type of soil	Treatment [1]	Ash from—	
				Kernels	Shells
				Percent	*Percent*
1	Small Virginia Runner.	Onslow fine sandy loam of lighter texture.	300 pounds of gypsum applied Aug. 1, 1933.	2.37	2.78
2	Jumbo Virginia Runner.	do	300 pounds of gypsum applied Aug. 1, 1933, followed by 40 pounds of K_2O from kainite at planting.	2.56	4.04
3	do	Onslow fine sandy loam, low spot of heavy texture.	300 pounds of gypsum applied Aug. 1, 1933, followed by 143 pounds of cottonseed meal at planting.	2.31	2.73
4	do	Onslow fine sandy loam of lighter texture.	300 pounds of gypsum applied Aug. 1, 1933 (check).	2.41	3.35
5	do	Onslow fine sandy loam of heavier texture.	1 ton of ground oystershell lime applied Apr. 1, 1934, followed by 3 bordeaux applications on July 15, Aug. 1 and 15, 1934.	2.22	2.89
6	do	do	1 ton of ground oystershell lime applied Apr. 1, 1934 (check).	2.46	4.00

[1] Amounts designated in pounds represent rates per acre.

Because of the comparatively small quantities of ash obtained from the peanuts of each individual plot, the ash portions from all plots were combined and mixed for qualitative examination, the results of which are recorded in table 4.

TABLE 4.—*Qualitative examination of ash from peanut kernels and shells*

Cation or anion	Relative amount in—	
	Kernels	Shells
Cation:		
Potassium	Very much	Much.
Sodium	Very little	Very little (trace).
Magnesium	Considerable quantity	Present.
Calcium	Present in smaller quantity than magnesium.	Present; much more than in kernels.
Iron	Present	Do.
Aluminum	do	Present in greater quantity than iron.
Anion:		
Phosphate	Considerable quantity	Present; very much less than in kernels.
Sulphate	Present	Present; somewhat more than chlorine.
Silicate	Not present	Present in considerable quantity.
Chlorine	Present	Present (trace).

It will be seen that calcium occurs in the shells in considerably greater quantity than in the kernels and that silicon is present in the shells only. On the other hand, there is more potassium, magnesium, and phosphorus in the seeds than in the shells.

ETHER EXTRACT

Duplicate 10-g portions of ground peanut kernels and shells, after being dried at 100° C., were extracted with anhydrous ether in a Soxhlet extraction apparatus for 5 to 6 hours, the results thus obtained being designated as analyses A (table 5). The residue was then dried, taken out of thimbles, reduced to a fine powder, returned to the thimbles, and extracted for 5 more hours. This was done in the case of the kernels because originally they had been ground somewhat

coarse. For uniformity in procedure the shells were also extracted in two stages, though the material was fine and did not need to be ground again. The results of the first and second extractions combined were designated as analyses B, table 5.

TABLE 5.—*Percentage of ether-soluble substances in peanut kernels and shells, determined on moisture-free basis*

Plot No.	Ether-soluble substances in—				Plot No.	Ether-soluble substances in—			
	Kernels		Shells			Kernels		Shells	
	Analyses A	Analyses B	Analyses A	Analyses B		Analyses A	Analyses B	Analyses A	Analyses B
	Percent	*Percent*	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*	*Percent*
1	46.70	46.91	0.55	0.57	4	46.86	48.02	0.56	0.60
2	46.77	47.42	.59	.61	5	47.92	48.59	.32	.38
3	48.41	49.41	.47	.48	6	47.25	48.49	.57	.57

In the case of the kernels the differences between analyses A and analyses B were quite pronounced, ranging from 0.21 percent (plot 1) to 1.24 percent (plot 6). This shows that analyses A do not represent the total ether-extractable material present in the kernels. For this reason, only analyses B, which can be considered as correct, are to be accepted. For the shells, A and B agree closely, but in five of the six samples the second stage of the extraction removed a slight additional amount of ether-soluble substances. The percentage of ether-extractable substances of the kernels from plot 3 (heavy soil and high organic matter) appears definitely greater than those from the comparable plots 2 and 4, whereas the reverse is true of the ether-extractable substances of the shells, an observation that merits further study. It seems also an interesting fact that the percentage of ether-extractable substances of the shells from the very vigorous plants of the bordeaux-treated plot was quite low in comparison with the control.

TOTAL NITROGEN AND PROTEIN

The total nitrogen was estimated by Gunning's modification of the Kjeldahl method. These determinations are designated in table 6 as analyses A. To make certain whether nitrate nitrogen was present, the oxidation of the peanut materials was effected also in the presence of salicylic acid (1 g of salicylic acid to 30 cc of sulphuric acid) and sodium thiosulphate, according to the method of the Association of Official Agricultural Chemists (*1, p. 21*). These determinations are designated as analyses B.

The percentage of protein was computed by multiplying the percentage of nitrogen by the factor 5.5. This factor was used by Jones and Horn (*9*) because Johns and Jones (*6, 7, 8*) had shown that the peanut contains two dominant proteins, the globulins arachin and conarachin, which contain 18.29 percent of nitrogen and which make up more than four-fifths of the total nitrogen of the peanut. The factor 5.5 was likewise employed by Ritthausen (*12*). The results thus obtained (table 6) are only approximations of the true protein content, for almost certainly some nonprotein nitrogen was present.

TABLE 6.—*Percentage of total nitrogen and of calculated protein in peanut kernels and shells, determined on moisture-free basis*

Plot No.	Kernels			Shells		
	Nitrogen by Kjeldahl method, analyses A	Nitrogen by Kjeldahl method modified to include nitrates, analyses B	Protein, average of analyses (A and B) ×5.5	Nitrogen by Kjeldahl method, analyses A	Nitrogen by Kjeldahl method modified to include nitrates, analyses B	Protein, average of analyses (A and B) ×5.5
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	4.88	4.89	26.87	1.56	1.60	8.69
2	4.99	5.06	27.64	1.11	1.14	6.19
3	4.90	4.91	26.98	1.21	1.24	6.74
4	4.96	4.92	27.17	1.55	1.60	8.66
5	4.63	4.71	25.69	1.09	1.13	6.11
6	4.91	5.02	27.31	1.51	1.43	8.09

Table 6 shows that the total nitrogen as determined to include nitrates in the kernels (analyses B) is in general insignificantly higher than the corresponding Kjeldahl nitrogen values. This is true for the shells also, the average total nitrogen content, including nitrates, being only 0.8 and 1.5 percent higher than the corresponding Kjeldahl nitrogen values in the case of the kernels and the shells respectively. In other words, the amount of nitrates, if at all present in the kernel and the shell, is so small as to be negligible.

Among the samples of kernels and shells there were no very striking differences in nitrogen content that could be definitely associated with the environment or growth of the plant. It is suggestive, however, that both kernels and shells from plot 5 showed a distinctly lower nitrogen content than the corresponding samples from plot 6. Although less striking, the nitrogen content of kernels of samples from plot 3 tended to be low as compared with that for plots 2 and 4. The differences among the shell samples were greater than among the kernel samples both relatively and absolutely.

CRUDE FIBER

The method of determining the crude fiber was essentially that of the Association of Agricultural Chemists (*1, pp. 280–281*). The results obtained (table 7) show that the differences in the crude-fiber content of the kernels from plots 2 to 6 (Jumbo Virginia Runner) were rather insignificant and that the value for plot 5 was the lowest. The differences in the crude-fiber content of the shells were marked, the value for plot 3 (heavy soil, high organic matter) being the highest, and that for plot 5 (bordeaux-treated) the lowest.

TABLE 7.—*Percentage of crude fiber in peanut kernels and shells, determined on moisture-free basis*

Plot No.	Crude fiber in—		Plot No.	Crude fiber in—		Plot No.	Crude fiber in—	
	Kernels	Shells		Kernels	Shells		Kernels	Shells
	Percent	*Percent*		*Percent*	*Percent*		*Percent*	*Percent*
1	2.82	65.42	3	2.20	66.23	5	2.11	61.63
2	2.21	63.46	4	2.24	63.13	6	2.23	62.62

CARBOHYDRATES

A careful review of the literature failed to reveal any estimations of reducing sugars, sucrose, or starch in the peanut kernel. Instead, nitrogen-free extract is ordinarily given, which is tacitly assumed to comprise the carbohydrates. The nitrogen-free extract is obtained by subtracting from the dry matter the sum of protein, crude fiber, ether extract, and ash. This indirect method is very convenient, and therefore is generally used in reporting analyses of feedstuffs, although it naturally involves the errors incidental to the determination of the afore-mentioned constituents and gives no knowledge of the specific carbohydrates present. Direct determinations of certain of the various carbohydrates are therefore necessary in order better to understand the physiological conditions associated with various types of response of the various parts of the peanut plant. The methods employed were as follows: 2 g of ground peanut kernels, first freed from fat by treatment with ether, were extracted with 60-percent alcohol in a Soxhlet extraction apparatus; the extract was freed from alcohol, and the aqueous solution was then introduced into a 250-cc volumetric flask; 1 to 2 cc of neutral lead acetate was added, and the mixture was made up with water to 250 cc and filtered clear.

DETERMINATION OF REDUCING SUGARS

Briefly stated, a 100-cc portion of the clear filtrate was boiled with Bertrand's solution A (copper sulphate) and B (Rochelle salt) for 3 minutes. The cuprous oxide obtained, after being washed well with hot water was dissolved in Bertrand's solution C (ferric sulphate) and titrated with Bertrand's solution D (standard potassium permanganate), the results being calculated as glucose (*3*, *13*, *14*).

DETERMINATION OF TOTAL SUGARS

A 75-cc portion of the clear filtrate was inverted according to Herzfeld's method (*4*). The cooled hydrolysate was neutralized with sodium hydroxide solution and made up to 100 cc. This, divided into two 50-cc portions, was used for the estimation of total sugar according to Bertrand's method, the results being calculated as invert sugar.

CALCULATION OF SUCROSE

The proportion of sucrose was found by subtracting the percentage of reducing sugars from that of total sugars and multiplying the difference by the factor 0.95.

ESTIMATION OF ACID-HYDROLYZABLE POLYSACCHARIDES

The estimation of the acid-hydrolyzable polysaccharides was made according to the method as outlined by Schmidt (*15*, *p. 924*), Lohrisch (*10*, *p. 375*), and Zemplén (*18*). The residue that remained after 2 g of peanut kernels had been extracted with ether and with 60-percent alcohol was hydrolyzed with a 2.1-percent hydrochloric acid solution for 2 hours. The cooled hydrolysate, after being neutralized with sodium hydroxide solution, was made up with water to 250 cc and filtered clear. Of this solution, 10-cc portions were used for deter-

mining the glucose according to Bertrand's method, the acid-hydrolyzable polysaccharides being calculated as starch by multiplying the glucose value obtained by the factor 0.9. For the details concerning the estimation of reducing sugars, sucrose, and hydrolyzable polysaccharides, the reader is referred to a previous publication (*5, pp. 716, 718*). The data secured are summarized in table 8.

TABLE 8.—*Percentages of reducing sugars, sucrose, and starch in the peanut kernel, determined on moisture-free basis*

Plot No.	Reducing sugars	Sucrose	Hydrolyzable polysaccharides	Total carbohydrates	Plot No.	Reducing sugars	Sucrose	Hydrolyzable polysaccharides	Total carbohydrates
	Percent	*Percent*	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*	*Percent*
1	0.25	2.45	6.98	9.68	4	0.26	2.67	5.35	8.28
2	.26	3.05	5.22	8.53	5	.27	3.18	5.70	9.15
3	.22	2.87	4.88	7.97	6	.28	3.05	4.78	8.11

Table 8 shows that the percentage of reducing sugars is very small. On the other hand, the proportions of sucrose are quite noticeable. Still larger are the proportions of hydrolyzable polysaccharides, and the percentages of the total carbohydrates range from 7.97 (plot 3) to 9.68 (plot 1). These values indicate that because of the vigorous vegetative growth of the plants in plot 3, on a heavier soil high in organic matter, the maturity of the kernels was retarded. As a consequence, the percentage of hydrolyzable polysaccharides (4.88) and of total carbohydrates (7.97) was noticeably smaller than in the comparable plots 2 and 4, which showed, respectively, a starch percentage of 5.22 and 5.35, and a total carbohydrate percentage of 8.53 and 8.28. The bordeaux-sprayed plants of plot 5, which exhibited a leaf color and leaf area far superior to the unsprayed plants of plot 6, also showed a higher content of sucrose and hydrolyzable polysaccharides—in fact, the highest of any plot of the Jumbo Virginia Runner. It is also noteworthy that the kernels of the Small Virginia Runner (plot 1) displayed the highest percentage of hydrolyzable polysaccharides (6.98) and of total carbohydrates (9.68), doubtless owing largely to the variety.

SUMMARY

Six samples of peanut fruits involving two varieties were taken from areas and soil-treatment plots of an experimental field showing marked differences in growth, yield, and character of fruits. These were subjected to partial chemical analysis to determine whether easily recognizable differences in composition might be associated with environment and growth response.

The two varieties showed slight differences in ash, oil, protein, crude fiber, and carbohydrate content, but too few samples were involved to permit the drawing of any definite conclusions.

Ash content appeared to be decreased in both kernels and shells by a high nitrogen supply of the soil or by bordeaux mixture treatment that resulted in markedly increased plant growth and yield of pods.

Relatively unimportant differences in ether extract of kernels were observed among the samples, although in the shells the percentage

of ether-extractable substances was apparently decreased by high nitrogen or by the bordeaux treatment.

The total nitrogen content of kernels and shells apparently was not influenced by a high nitrogen condition of the soil due to application of cottonseed meal, but the nitrogen percentage was less in both kernels and shells from the bordeaux-treated plot.

Differences in crude fiber among the samples were very small and of doubtful importance.

Reducing-sugar content was extremely low in all samples of kernels. The sucrose content of kernels from the high-nitrogen plot treated with cottonseed meal and that for the bordeaux-treated plot were not materially different from their corresponding checks.

Total polysaccharides and total carbohydrates appeared to be decreased somewhat on the high-nitrogen plot and increased on the bordeaux-treated plot, in comparison with their respective checks.

This preliminary examination suggests that further biochemical and physiological studies may be expected to reveal important information on growth and development problems relating to yield and quality of peanuts.

LITERATURE CITED

(1) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.
1930. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS . . . Ed. 3, 593 pp., illus. Washington, D. C.

(2) BEATTIE, W. R.
1920. PEANUT GROWING FOR PROFIT. U. S. Dept. Agr. Farmers' Bull. 1127, 33 pp., illus.

(3) BERTRAND, GABRIEL.
1906. LE DOSAGE DES SUCRES RÉDUCTEURS. Bull. Soc. Chim. France (3) 35: 1285–1299.

(4) HERZFELD, ALEXANDER.
1888. DIE ART DER AUSFÜHRUNG DER INVERSIONSMETHODE. Ztschr. Ver. Rübenzucker Indus. 38: 699–722.

(5) JODIDI, SAMUEL L., and BOSWELL, VICTOR R.
1934. CHEMICAL COMPOSITION AND YIELD OF THE ALASKA PEA AS INFLUENCED BY CERTAIN FERTILIZERS AND BY THE STAGE OF DEVELOPMENT. Jour. Agr. Research 48: 703–736.

(6) JOHNS, CARL O., and JONES, D. BREESE.
1916. THE PROTEINS OF THE PEANUT, ARACHIS HYPOGÆA. I. THE GLOBULINS ARACHIN AND CONARACHIN. Jour. Biol. Chem. 28: 77–87.

(7) ——— and JONES, D. BREESE.
1917. THE PROTEINS OF THE PEANUT, ARACHIS HYPOGÆA. II. THE DISTRIBUTION OF THE BASIC NITROGEN IN THE GLOBULINS ARACHIN AND CONARACHIN. Jour. Biol. Chem. 30: 33–38.

(8) ——— and JONES, D. BREESE.
1918. THE PROTEINS OF THE PEANUT, ARACHIS HYPOGÆA. III. THE HYDROLYSIS OF ARACHIN. Jour. Biol. Chem. 36: 491–500.

(9) JONES, D. BREESE, and HORN, MILLARD J.
1930. THE PROPERTIES OF ARACHIN AND CONARACHIN AND THE PROPORTIONATE OCCURRENCE OF THESE PROTEINS IN THE PEANUT. Jour. Agr. Research 40: 673–682.

(10) LOHRISCH, HANS.
1911. METHODEN ZUR UNTERSUCHUNG DER MENSCHLICHEN FÄZES. *In* Abderhalden, Emil, Handbuch der Biochemischen Arbeitsmethoden, Bd. 5, Teil 1, pp. [331]–420, illus. Berlin and Wien.

(11) MOORE, RUFUS H.
1937. NUTRITIONAL LEVELS IN THE PEANUT PLANT. Bot. Gaz. 98: 464–490, illus.

(12) RITTHAUSEN, H.
1880. ÜBER DIE EIWEISSKÖRPER VERSCHIEDENER ÖLSAMEN. Pflüger's Arch. Physiol. 21: 81–104.
(13) ROSENBLATT, M.
1912. ÜBER DIE QUANTITATIVE BESTIMMUNG VON GLUCOSE BEI GEGENWART VON FREMDEN STOFFEN NACH DER ANALYTISCHEN METHODE VON GABRIEL BERTRAND. Biochem. Ztschr. 43: [478]–480.
(14) ———
1913. BEMERKUNG ZU EINER MITTEILUNG VON G. SONNTAG: "DIE METHODE VON GABRIEL BERTRAND ZUR ZUCKERBESTIMMUNG." Biochem. Ztschr. 57: [335]–336.
(15) SCHMIDT, ERNST.
1910. AUSFÜHRLICHES LEHRBUCH DER PHARMAZEUTISCHEN CHEMIE. Aufl. 5, Bd. 2, Abt. 1, 1020 pp., illus. Braunschweig.
(16) UNITED STATES DEPARTMENT OF AGRICULTURE.
1934. AGRICULTURAL STATISTICS. U. S. Dept. Agr. Yearbook 1934: 383–766.
(17) UNITED STATES TARIFF COMMISSION.
1929. PEANUTS, NOT SHELLED AND SHELLED. REPORT OF THE UNITED STATES TARIFF COMMISSION TO THE PRESIDENT OF THE UNITED STATES . . . 88 pp., illus. Washington, D. C.
(18) ZEMPLÉN, GÉZA.
1912. DARSTELLUNG, GEWINNUNG, NACHWEIS UND BESTIMMUNG DER HÖHEREN KOHLENHYDRATE. *In* Abderhalden, Emil, Handbuch der BiochemischenArbeitsmethoden, Bd. 6, pp. 1–82, illus. Berlin and Wien.

INFLORESCENCE BLIGHT OF THE DATE PALM[1]

By J. G. BROWN, *plant pathologist*, and KARL D. BUTLER, *research assistant*, *Arizona Agricultural Experiment Station*

INTRODUCTION

In 1935 a blight of the inflorescence of the date palm in university date garden at Tempe, Ariz., was called to the attention of the senior author. The disease was present on the Sayer and Khadrawi varieties. In 1936 Khadrawi palms were not attacked, but the Khir variety, hitherto not observed to be affected, had flower clusters killed by the blight. A survey [2] of the palms showed the following blighted inflorescences, all pistillate:

Variety:	*Blighted inflorescences*
Iteema	1
Maktoom	2
Sayer (31 years old)	7
Sayer (31 years old)	5
Sayer (11 years old)	1
Khir (31 years old)	4

The occurrence of the blight in the date garden suggested the advisability of examining the date palms on the campus of the University of Arizona at Tucson. A survey of these palms showed the disease to be present on staminate inflorescences.

The date palms with which the writers worked in university date garden are chiefly known varieties imported from Africa and propagated by means of offshoots. The history of the garden is given in Arizona Agricultural Experiment Station Bulletin 149 (*1*).[3] Palms on the campus of the University of Arizona, which are mentioned in connection with the present study, are seedling in origin and nondescript in variety, and were planted primarily for ornamental purposes.

Until 1936 inflorescences infected with blight fungi had been found only on palms 30 to 31 years old, but during the flowering season of 1936 an inflorescence blighted on a palm only 11 years old. Most infected inflorescences in the date garden have been found on pistillate flowers, but blighted staminate inflorescences have also been collected there and on the university campus. Hilgeman [4] states that a blight which occurs generally on staminate flower clusters in the date garden and throughout the Salt River Valley in Arizona has been popularly attributed to *Thielaviopsis paradoxa* on the basis of work done on palms in California (*4*). The present work (the first investigation of inflorescence blight to be undertaken at this station) appears to indicate that more or less of the blight in Arizona date palm orchards is caused by fusaria.

[1] Received for publication February 28, 1938; issued August 1938.
[2] This survey was made by Robert H. Hilgeman, assistant horticulturist.
[3] Italic numbers in parentheses refer to Literature Cited, p. 318.
[4] Personal communication.

SYMPTOMS

Initial infection of date inflorescences may occur anywhere on the rachis from the base to the branches, on the branches, or in the flowers. Primary infection of the rachis results in water soaking of the tissues and subsequent discoloration of the stalk, the color ranging from wood brown (*6*) to fawn to bone brown, with shrinking of the infected tissues (pl. 1, *A*, *a*). Disease of the branches, which are discolored like the rachis, results in the death of the flowers above the infected spot. Attacked flowers (pl. 1, *E*, *F*) are a discolored brown.

Stalks of pistillate inflorescences often are so weakened by infection that they break under the increasing weight of the fruit. The break across the stalk is at right angles to the axis, often resembles a cut made by a sharp instrument, and is deep enough to permit the distal part of the branch above the break to bend back against the basal part. Although this breaking of the stalk following infection is common, apparently sound stalks occasionally break. The writers have cultured some that gave no parasitic organisms.

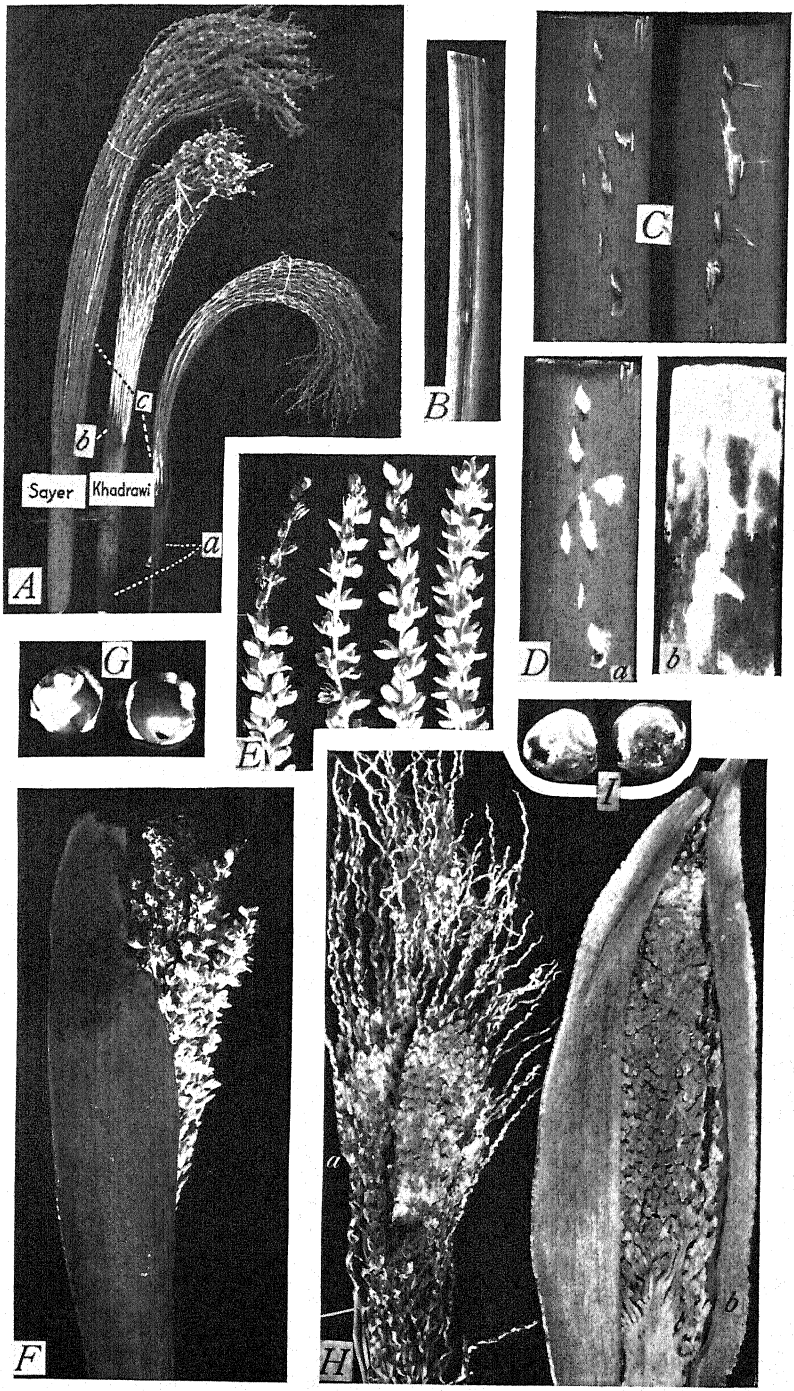
CAUSE

Blighted parts of the date inflorescence, sometimes in the field and regularly in the laboratory when kept moist, become overgrown with a gray to buff mycelium, or with a covering of pale purplish to pale Congo pink filaments, or with a mycelium in which these colors are mixed. Blocks of tissues from infected parts, which were surface-sterilized and cultured on 2-percent potato-dextrose agar, steamed yellow corn meal, and steamed rice, yielded two species of *Fusarium* and one species of *Helminthosporium*. One *Fusarium*, in 10-day cultures on potato-dextrose agar, was a pale grayish vinaceous above and pale grayish vinaceous to brownish vinaceous in streaks below. On steamed rice in 6-day cultures it was light vinaceous gray above and light vinaceous lilac with rocellin purple lines around the lighter areas below.

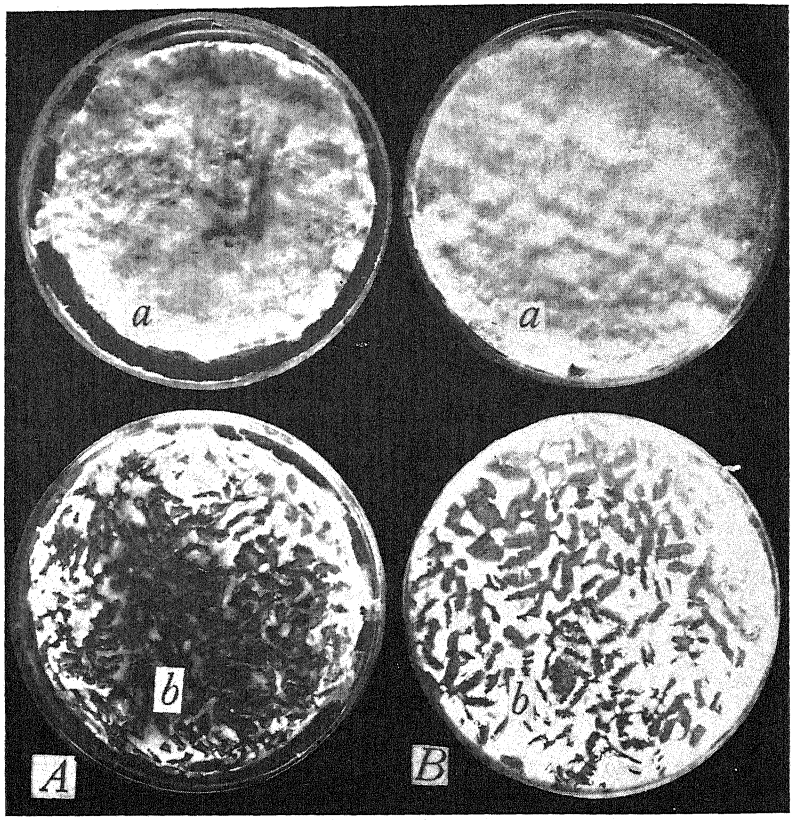
The other species of *Fusarium*, in 2-day cultures on potato-dextrose agar, was sea-shell pink to salmon buff above, and gray to ochraceous orange (margin of colony to center) below; 10-day cultures on the same medium were pale ochraceous salmon to salmon buff above (the lighter color in the margin and between the concentric growth rings), and gray to orange below. On steamed rice, 9-week cultures were pinkish buff above and pinkish buff to hazel below. This species often gave concentric growth rings 2 to 4 mm wide on potato-dextrose

EXPLANATORY LEGEND FOR PLATE 1

A, Naturally blighted inflorescence of date palm, Khadrawi variety, with: *a*, Basal discoloration and shrinking of rachi; *b*, discoloration of branches; *c*, mycelium and spore masses of *Fusarium*. *B*. Piece of rachis inoculated in situ with *Fusarium moniliforme* on April 21, 1935, and photographed May 24, 1935. Discoloration of the rachis extends for a considerable distance above and below the points of inoculation. *C*, Pieces of rachis of date palm laboratory-inoculated with *F. moniliforme* April 21, 1935, and photographed May 4 following. *D*, Pieces of rachis laboratory-inoculated with *F. moniliforme* April 21, 1935, and photographed May 15: *a*, Piece incubated at 5° C.; *b*, piece incubated at room temperature. *E*, Flowers of seedling date palm inoculated with *F. moniliforme* April 21, 1935, and photographed May 4. *F*, Natural infection of the staminate inflorescence of seedling date palm with *F. moniliforme* May 24, 1935; terminal flowers discolored and killed. *G*, Green date fruits inoculated by spraying with a spore suspension of *F. moniliforme;* photographed 7 days after inoculation. *H*, Natural infection of staminate inflorescence of date palm with *F. semitectum* (*F. lateritium* var. *fructigenum*?), July 20, 1935: *a*, Late stage showing mycelium and spore masses on inflorescence in the university date orchard; *b*, early stage, campus, University of Arizona. *I*, Green date fruits inoculated by spraying with a spore suspension of *F. semitectum* (*F. lateritium* var. *fructigenum*?); photographed 7 days after inoculation.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.



A, Culture of *Fusarium moniliforme* from blight of date inflorescence, on steamed rice 39 days at 28° C.: *a*, Upper surface, light vinaceous gray; *b*, lower surface light vinaceous lilac with rocellin purple borders around lighter areas. *B*, Culture of *Fusarium semitectum* (*F. lateritium* var. *fructigenum*?) from blight of date inflorescence, on steamed rice, 39 days at 28° C.: *a*, Upper surface, pale ochraceous salmon to salmon buff; *b*, lower surface gray to ochraceous orange.

agar. Plate 2, *A* and *B*, shows the appearance of older cultures of the two fusaria.

The vinaceous culture of *Fusarium* was identified by Dr. C. D. Sherbakoff, of the Tennessee Agricultural Experiment Station, as *Fusarium moniliforme* Sheld., which agreed with the report on the same fungus received from the Centraalbureau voor Schimmelcultures, Baarn, Netherlands. The buff culture was identified by Sherbakoff as *Fusarium semitectum* Berk. et Rav. and by the Centraalbureau as *Fusarium lateritium* Nees var. *fructigenum* (Fr.) Wr.

The species of *Helminthosporium* found by the junior author and by him proved capable of causing decay of the flower clusters of the date palm is not considered in the present paper.

INOCULATIONS

On April 19, 1935, three spadices of *Phoenix dactylifera L.*, whose spathes had been open a few days, were inoculated by spraying the exposed flowers with a heavy suspension of spores of *Fusarium moniliforme;* and a small hole was cut through an unopened bract of a fourth inflorescence into which a spore suspension of the fungus was injected, so that the spathe formed a natural moist chamber. On the same date four rachi were inoculated by inserting small pieces of a pure culture of the fungus from potato-dextrose agar into small incisions made with a sterile scalpel and afterward moistened with sterile distilled water. Both staminate and pistillate inflorescences were used in the inoculations. On May 4 many diseased flowers were evident in the inoculated clusters (pl. 1, *E*), but the controls were normal. A series of 12 isolations was made from the inoculations, and all but 1 culture gave the *Fusarium* species used in the inoculations of the inflorescences. Too rigorous surface sterilization of the tissues used in culture may account for the single failure.

Again on April 21, 10 rachi on 2 palms were wound-inoculated as previously described; 13 days later the inoculations had water-soaked margins and presented the appearance shown in plate 1, *C*, although the controls showed no infection. Pieces of the water-soaked tissues were surface-sterilized with a 1:1,000 mercuric chloride solution and cultured, with the recovery of *Fusarium moniliforme* in 11 of the 15 tubes.

Two large pieces of rachi, which were scalpel-inoculated with *Fusarium moniliforme* and placed under a bell jar in the incubator at 27° C., showed good fungus growth on the wounds in 3 days, although no growth occurred on control pieces similarly wounded but not inoculated. One piece was then transferred to the refrigerator and kept at a constant temperature of 5° in a similar moist chamber. At the end of 8 days the two pieces were photographed (pl. 1, D, *a* and *b*). Growth at the lower temperature was slight, but at the higher temperature it was luxuriant.

The piece of rachis from the incubator was afterward removed to a moist chamber in the laboratory and there it produced numerous perithecia. The perithecia were dark blue, ovoid, contained asci with eight ascospores arranged in two series, and agreed well with the measurements of perithecia, asci, and ascospores given by Wollenweber (*7*) for *Gibberella moniliformis* (Sh.) Wineland. Ascospores from the perithecia were cultured on malt agar from which transfers

were made to potato-dextrose agar; the resulting mycelial growth appeared to be identical with *Fusarium moniliforme* and produced similar microconidia.

On May 24 a rachis which had been inoculated with a culture of *Fusarium moniliforme* on April 21 was found to have a long, depressed, grooved lesion (pl. 1, *B*) which had resulted from the coalescence of the lesions initiated by inoculation. Blocks of the infected tissues from this piece of rachis were killed and sectioned for histological study.

On May 24 a natural infection of a staminate inflorescence was found on a date palm on the university campus. The spathe had been open for a few days only, yet the top of the flower cluster was very brown and the infection appeared to be progressing downward (pl. 1, *F*). From cultures of the infected flowers *Fusarium moniliforme* was isolated.

Inoculations of date palm parts with *Fusarium semitectum* (*F. lateritium* var. *fructigenum*, according to the Centraalbureau voor Schimmelcultures) were made as follows: Wound inoculations on five rachi and two petioles and spore-suspension sprayings on two inflorescences, all made on May 15 with proper controls. On June 15 lesions were present on all inoculated parts except the petioles, but none were present on controls; the lesions developed much more slowly than those caused by inoculation with *F. moniliforme*. From the inoculated tissues 20 cultures were made with surface-sterilized pieces; 12 of the cultures gave the *Fusarium* used in the inoculations—the only pathogene recovered. Green, surface-sterilized date fruits from palms on the campus, placed in Petri dishes, were vigorously attacked by *F. semitectum* (*F. lateritium*?) and by *F. moniliforme*, after being sprayed with spore suspensions of these species (pl. 1, *G*, *I*).

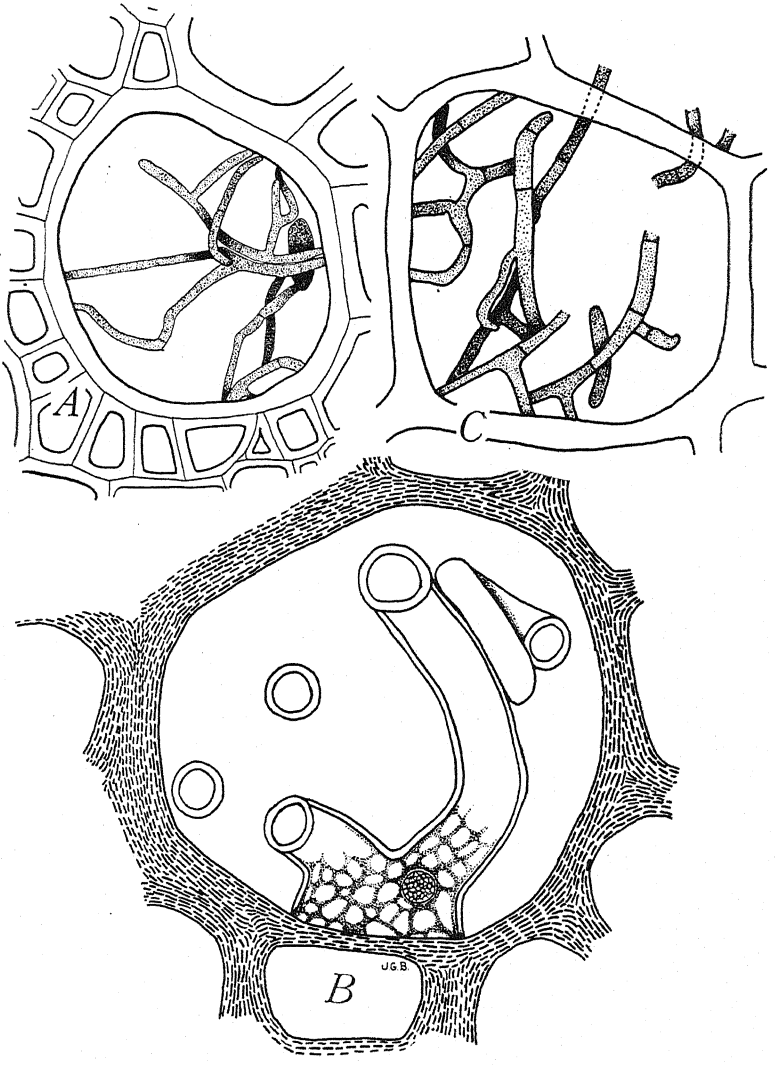
PATHOLOGIC HISTOLOGY

Preparations for microscopic investigation were made from the tissues of rachi and other parts infected with *Fusarium moniliforme*. The fungus infects both fibro-vascular bundles and parenchyma cells, which are more or less discolored as a result of invasion. In the bundles the filaments are located chiefly in the tracheae (pl. 3, *A* and *B*); in the parenchyma tissue they are both intercellular and intracellular (pl. 3, *C*). Infected parenchyma cells in slides stained with carbol fuchsin-light green (clove oil) acquired a deeper red than healthy cells in the same preparations. The vacuolar structure of the hyphae in the vessels was clearly evident under high magnification (pl. 3, *B*).

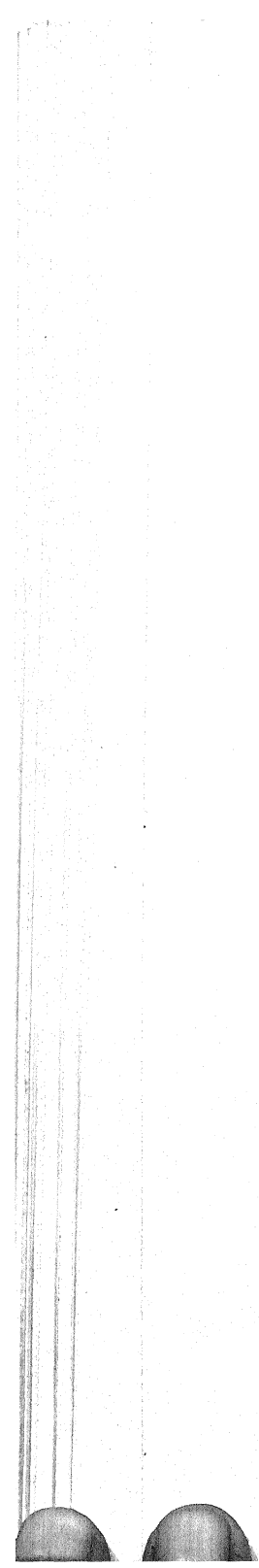
CONDITIONS AFFECTING THE BLIGHT

Although a study of the effect of environmental conditions on the prevalence of the blight of date inflorescences has not been made, observations indicate that the weather in the Salt River Valley during the flowering season is usually favorable for its development; hence the general occurrence of the disease. Viable cultures of *Fusarium* from old inflorescences show that the heat of summer, the dryness of autumn, and the cold of winter do not injure the fungus, which continues capable of producing abundant spores.

How the spores of the fusaria of inflorescence blight are disseminated remains to be determined. Insects are frequently very numerous in



A, *Fusarium moniliforme:* Parts of hyphae in trachea of fibrovascular bundle of rachis. × 621.4. *B*, vacuolate structure of a part of a hypha of *F. moniliforme* in a trachea of date rachis. × 2,571. *C*, parts of hyphae of *F. moniliforme* in a parenchyma cell of rachis of date palm. × 621.4.



the tops of date palms, especially during flowering and fruiting. Possibly they are the important agents of dissemination. The practice among date growers of carrying flower clusters from staminate palms into their exclusively pistillate plantings no doubt facilitates the spread of the inflorescence blight.

The effect of spraying on fusarium blight of date flower clusters has not been specifically investigated, although the trees in university date garden have been sprayed for several years in an effort to control fruit rot. In these sprayings copper acetate, lime-sulphur, and instant bordeaux mixture (4–4–50) have been used. Sprays for fruit rot usually have been applied later in the season than they should be to control inflorescence blight. Perhaps the first step in the control of the blight should be the removal of all staminate flower clusters after pollination is effected, and the removal of all pistillate rachi after the fruit is harvested. With these sources of infection out of the way, a thorough spraying of the palms before the spathes break open should go far toward eliminating the blight.

DISCUSSION

In some respects the blight of date inflorescences herein discussed is similar to the decay described by Cavara (*2*), and later by Chabrolin (*3*), under the name "Khamedj," for palms in north Africa. Both diseases are parasitic, both attack staminate and pistillate flower clusters and related parts, both originate externally, and both cause browning of the affected tissues. Also, the Khamedj parasite has been proved to attack uninjured tissues just as the fusaria in inflorescence blight attacked uninjured green date fruits. However, there should be no possibility of confusing the Khamedj parasite with the fusaria, for the former, *Mauginiella scaettae* (*2, 3*), belongs to a genus having affinity with *Septocylindrium* and *Geotrichum;* it produces a white efflorescence, becoming pulverulent over reddish spots which later become black; the external mycelium disappears from the infected spots; and conidia are borne in chains. Except for a somewhat pulverulent appearance, the fusaria differ from the Khamedj parasite as described, and no pathologist would have difficulty in distinguishing the parasites.

The blight described in this paper may be the same inflorescence decay mentioned by Fawcett and Klotz (*4*) in their bulletin on diseases of the date palm, although they do not give the species of *Fusarium* which they isolated in their studies. They state (*4, p. 23*): "*Fusarium* sp. is sometimes found mixed with either *Thielaviopsis* or *Diplodia,* or it may be alone." In another publication (*5, p. 157*) they say: "A species of Fusarium was later found as the primary cause of a decay of certain male inflorescences of the date palm." The fact that they obtained less injury from their inoculations with *Fusarium* species may have been due to their use of a different species from those employed by the writers, or to different environmental conditions. Inoculation with *Fusarium moniliforme* in the writers' experiments soon resulted in extensive decay. From brief description of the *Fusarium* rot given by the California investigators it is impossible to determine whether the inflorescence decay which they mention is identical with the blight observed in the Arizona plantings.

That the blight may affect the inflorescences of a comparatively wide range of varieties and ages of date palms is indicated by the list given in the introduction to this paper, and this probably means a spread of the disease unless steps are taken for its control. Persistent blighted flower and fruit clusters may easily become serious sources of infection from year to year. One suspicious case found on the campus strongly suggests this means of carry-over. A rotted cluster of flowers from the preceding year, within a partly opened spathe, was covered and interspersed with a powderly mass of *Fusarium* spores; on the same palm several blighted inflorescences were found near the old blighted flower cluster.

SUMMARY

An inflorescence blight attacks several varieties and ages of date palms in southern Arizona.

Primary infection results in water soaking and discoloration of the infected parts; flowers may be killed by direct attack or indirectly by the infection of parts below them; green fruits decay when sprayed with a spore suspension of either of the fungi concerned.

Two species of *Fusarium* cause the blight, working together or separately. One of these is *Fusarium moniliforme* Sheld.; the other is either *F. semitectum* Berk. et Rav., (as identified by Sherbakoff) or *F. lateritium* Nees var. *fructigenum* (Fr.) Wr. (as indicated by Centraalbureau voor Schimmelcultures).

The results of numerous inoculations with the two species of *Fusarium* on different parts of the date palm are given.

A brief description of the pathologic histology is presented.

Conditions affecting the blight are discussed, and suggestions for its control are given.

LITERATURE CITED

(1) Albert, D. W., and Hilgeman, R. H.
1935. Date growing in Arizona. Ariz. Agr. Expt. Sta. Bull. 149, pp. 231–286, illus.

(2) Cavara, F.
1926. "Mauginiella scaettae" cav., nuovo ifomicete parassita della palma da datteri di cirenaica. Bull. Orto Bot. Univ. Napoli 8: [207]–210, illus.

(3) Chabrolin C.
1929. La pourriture de l'inflorescence de palmier-dattier (khamedj). Compt. Rend. Acad. Sci. [Paris] 188: 933–935.

(4) Fawcett, H. S., and Klotz, L. J.
1932. Diseases of the date palm, phoenix dactylifera. Calif. Agr. Expt. Sta. Bull. 522, 47 pp., illus.

(5) Klotz, L. J., and Fawcett, H. S.
1932. Black scorch of the date palm caused by thielaviopsis paradoxa. Jour. Agr. Research 44: 155–166, illus.

(6) Ridgway, Robert.
1912. Color standards and color nomenclature. 43 pp., illus. Washington, D. C.

(7) Wollenweber, H. W.
1931. Fusarium-monographie. Fungi parasitici et saprophytici. pp [269]–516, illus. Berlin. [Sonderabdruck aus Ztschr. Parasitenk. bd. 3, heft 3.]

U. S. GOVERNMENT PRINTING OFFICE: 1938

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 57 WASHINGTON, D. C., SEPTEMBER 1, 1938 No. 5

EXPERIMENTS ON THE TRANSMISSION OF POTATO VIRUSES BY VECTORS[1]

By T. P. DYKSTRA, *associate pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture,* and W. C. WHITAKER, formerly *research assistant, Department of Botany, Oregon Agricultural Experiment Station*[2]

INTRODUCTION

Virus diseases of potato (*Solanum tuberosum* L.) spread fairly rapidly in certain localities, whereas in others there is very little spread even in the same potato variety. In Maine (*7*)[3] mild mosaic spreads rapidly but rugose mosaic and leaf roll do not. Gardner and Kendrick (*8*) report that in Indiana leaf roll spreads more rapidly than mosaic. In Nebraska (*9*) spindle tuber infects a large number of plants unless special precautions are taken, but there is little spread of mild mosaic, rugose mosaic, and leaf roll.

It is assumed that the variation in the rate of spread in different localities is influenced by the abundance of certain species of insects, especially aphids, which are vectors of certain potato viruses. To secure definite information on the ability of different species of insects to transmit potato viruses, experiments were conducted in the field under muslin-covered cages and in the greenhouse. Since there also seems to be a variation among different species of aphids, which are known to be vectors, in their ability to transmit these viruses readily, and since infection of a plant with a virus may depend to some extent on the manner of application of the fluid containing the virus, it was considered important to determine the methods by which different species of aphids feed, and to try to correlate the feeding methods with the ability of the aphid to transmit a virus. The feeding habits on potato of four species of aphids, namely, *Myzus persicae* (Sulz.), *M. circumflexus* (Buckt.), *M. solani* (Kalt.), and *Macrosiphum (Illinoia) solanifolii* (Ashm.), were studied. These were taken from plants infested with one species only and were kept caged in the field or in the greenhouse. An effort was also made to determine whether insects other than aphids that naturally feed on potato plants may serve as vectors of potato viruses.

REVIEW OF LITERATURE

Schultz et al. (*19*) were the first to demonstrate that at least two species of aphids can transmit potato mosaic. The spread of the leaf roll virus of potato in the field from affected to healthy plants by means of aphids was first established by Oortwijn Botjes (*15*). This finding was confirmed independently by Schultz and Folsom (*16*). The

[1] Received for publication December 18, 1937; issued September 1938. This paper is based upon investigations carried on as part of a cooperative project between the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Oregon Agricultural Experiment Station.

[2] The authors wish to express appreciation to C. F. W. Muesebeck, principal entomologist in charge, Division of Insect Identification, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, for checking the scientific names of the insects.

[3] Italic numbers in parentheses refer to Literature Cited, p. 333.

successful transfer of leaf roll by means of *Myzus persicae* from and to sprouts was reported by Murphy (*12*).

Murphy and McKay (*14*) presented evidence that capsids (*Calocoris norvegicus* (Gmel.) (=*bipunctatus* F.)) and jassids (*Typhlocyba ulmi* (L.)) could also transmit leaf roll.

Elze (*6*) experimented with leaf roll, common mosaic, crinkle, stipple streak, interveinal mosaic, aucuba mosaic, and spindling sprout. With these diseases infections were carried out with *Myzus persicae*, *M. solani*, *Aphis rhamni* Boyer, *A. fabae* Scop., *Eupteryx auratus* (L.) *Lygus pratensis* (L)., *Psylliodes affinis* Payk., and *Mamestra brassicae* (L.). Elze claims that every one of these species possesses the power to transmit one or more of these diseases, with the exception of aucuba mosaic. Leaf roll was the easiest to transmit. He found that infection was always more difficult to accomplish with insects other than aphids. There were also differences between aphids, the infecting power of *Myzus persicae* being the greatest.

Smith (*23*) secured uniformly negative results when the following insects were tested as vectors of leaf roll from diseased to healthy sprouts: *Calocoris norvegicus* (=*bipunctatus*), *Lygus pabulinus* (L.), *Eupteryx auratus*, *Empoasca viridula* (Fall.), *Psylliodes affinis*, and *Macrosiphum* (*Illinoia*) *solanifolii*. He found, however, that leaf roll was transmitted with great regularity by *Myzus persicae*. In a later paper Smith (*24*) reported that a noninfective aphid can pick up the virus of leaf roll from an infected potato plant after 6 hours of feeding. The infective aphid is capable of transmitting the leaf roll virus to a healthy potato plant after 2 hours of feeding. However, the whole process cannot be performed in 8 hours; a minimum period of approximately 54 hours appears to be necessary. Smith also secured positive results with *Myzus solani* and the greenhouse aphid *M. circumflexus*, but with *Macrosiphum* (*Illinoia*) *solanifolii*, *Aphis rhamni*, and *A. gossypii* Glover his results were negative.

Cleveland (*2*) reported that *Myzus persicae* and the potato leafhopper (*Empoasca fabae* (Harr.)) are the principal insect carriers of potato leaf roll in Indiana, and he concluded that they are about equally responsible. He found that the potato flea beetle (*Epitrix cucumeris* Harris), *Thrips tabaci* Lind., blister beetles, Colorado potato beetles, grasshoppers, and white flies did not transmit the disease.

Schultz and Folsom (*17*) obtained mild mosaic in experiments with *Macrosiphum* (*Illinoia*) *solanifolii* and *Aphis abbreviata* Patch, but with flea beetles and Colorado potato beetles their results were negative.

McKay and Dykstra (*11*) failed to convey mild mosaic by means of *Macrosiphum* (*Illinoia*) *solanifolii*, *Myzus persicae*, *M. solani* (=*pseudosolani* Theob.), and *M. circumflexus* in 53 attempts. They also failed to convey rugose mosaic by means of *M. solani* and *Macrosiphum* (*I.*) *solanifolii* in 100 attempts, but they were able to convey it occasionally by means of *Myzus persicae* and *M. circumflexus*. Crinkle mosaic was not transmitted by *Macrosiphum* (*I.*) *solanifolii* and *Myzus solani* in 35 tests, but it was transmitted by *M. persicae* and by *M. circumflexus*. Leaf roll was readily transmitted by *M. solani*, *M. persicae*, and *M. circumflexus*, but only occasionally by *Macrosiphum* (*I.*) *solanifolii*. Murphy and McKay (*13*) reported similar

results when they stated that with this latter species only 1 out of 151 plants became infected with leaf roll.

Schultz and Folsom (*17*) reported successful virus disease infections by means of *Myzus persicae*, *Aphis abbreviata*, and *Macrosiphum (Illinoia) solanifolii*. They found that the last-named species transmitted mild mosaic both alone and in combination with leaf roll. In 1925 these investigators (*18*) experienced difficulty with aphid inoculation and stated that aphids sometimes do not transmit disease under conditions that apparently are the same as those that usually give positive results.

Murphy and McKay (*13*) are of the opinion that several species of aphids are transmitters of mosaic, but they found that under apparently similar conditions inoculation was often not successful. They state (*13, p. 152*):

> We have at different times successfully conveyed mosaic and leaf-roll infection from tuber to tuber in this way [by means of aphids on the sprouts], but on many occasions the results have been mainly or entirely negative. * * * Three different aphids have been used at various times, these being *Myzus persicae*, *M. pseudosolani* [*M. solani*], and *Macrosiphum solanifolii*. All have carried infection at times, so the conflicting results do not depend on the sort of aphis used; * * * the length of their stay on the source of infection or on the variety of plants being infected * * *.

Smith (*22*) also concluded that under certain conditions the aphids *Myzus persicae* and *Macrosiphum (Illinoia) solanifolii* can act as efficient transmitters of potato mosaic. He states: "In the present state of our knowledge of this subject it is not possible to say what these conditions may be."

From healthy potato plants Dykstra (*4*) collected miscellaneous insects, consisting of *Myzus persicae*, *Macrosiphum (Illinoia) solanifolii*, *Epitrix subcrinata* Lec., *Empoasca filamenta* De L., *Nabis alternatus* Parsh., *Lygus pratensis*, and *Philaenus leucophthalmus* (L.), and introduced them into two large cages, each covering about 80 potato plants. In one cage there was planted as a source of infection 10 solanaceous weeds infected with leaf roll, and in the other, 8 infected with rugose mosaic. The following year the tubers from each potato plant were planted as hill lots, and it was found that 82 percent of the plants from the first cage had become infected with leaf roll and 53 percent of those from the second cage showed typical rugose mosaic. This demonstrated that insects naturally occurring on potatoes are able to transmit these two viruses from infected weeds to healthy potatoes.

Smith (*21*) studied the feeding habits of three species of aphids, namely, *Myzus persicae*, *M. circumflexus*, and *Macrosiphum (Illinoia) solanifolii*. The stylet tracks of these aphids were intercellular and directed to the phloem, though occasionally the stylet track of *M. (I.) solanifolii* was intracellular.

Davidson (*3*) found that the piercing organ of *Aphis rumicis* L. passes intercellularly through the cortex, only occasionally passing through individual cells, until eventually it reaches the vascular bundles.

Büsgen (*1*) studied the method of feeding of hemipterous insects and the particular region of plant tissue sought by them. He found for aphids three types of stylet track, namely, intercellularly to the phloem, intracellularly to the phloem, and intracellularly through the parenchyma with no phloem objective.

PROCEDURE FOLLOWED IN TESTING INSECTS AS VECTORS OF POTATO VIRUSES

The studies on insect transmission were conducted in the greenhouse (fig. 1) and in the field under muslin-covered cages at the Agricultural Experiment Station, Corvallis, Oreg. The cages were set in position at the time the potato seed pieces were planted. This precaution was taken to prevent any insects not harbored in the soil, which might be virus carriers, from gaining access to the plants at any stage in their development. Each cage was large enough to cover three full-grown plants. In the inoculation work the cages employed for each test were grouped in units of four. Three healthy potato tubers of the same variety and origin were used in planting each group of cages. The first tuber was cut into four seed pieces, and these were planted as hill 1 in each of the four cages. Hills 2 and 3 were planted in the same manner. When the plants were 3 to 5 inches high, a species of aphid or other insect was transferred from diseased potato plants to each of the first three cages of a unit; the plants in the fourth cage were left uninoculated and kept as controls. If the control plants became infected with a virus the results from the plants in the first three cages of the unit were discarded.

APHIDS

Four species of aphids, *Macrosiphum* (*Illinoia*) *solanifolii*, *Myzus persicae*, *M. solani* (=*pseudosolani*), and *M. circumflexus*, were tested as agents of spread for the different virus diseases under test.[4] The first three are commonly found in potato fields in Oregon, but the fourth, so far as known, does not ordinarily occur on potatoes naturally, but when transferred to them, colonizes readily.

To provide aphids for infecting plants in the field in the spring, tubers known to be infected with various viruses were planted in 6-inch pots in the greenhouse and covered with individual muslin-covered cages. After the plants were about 6 inches tall a number of individuals of one of the four species of aphids used were taken from healthy plants and placed on the caged diseased plants and permitted to colonize. After a large colony of aphids had formed on a diseased plant about 50 were transferred by means of a camel's-hair brush to healthy plants 4 to 6 inches high growing under cages in the field; in some cases a leaf on which many aphids were feeding was placed on top of the plant under the cage. A week later all caged plants to which aphids had been transferred were examined, and if the insects had failed to colonize in a particular cage a new supply from a similarly infected greenhouse plant was introduced. In practically all cases vigorous colonies developed. Three weeks after the aphids had been introduced the plants in the field cages were dusted with 10-percent nicotine sulphate to kill the aphids and prevent them from stunting the plants.

In 1933 a diseased tuber, in some cases infected with both mosaic and leaf roll virus, was planted in the center of the cage in a 6-inch pot. At each end of the cage a healthy seed piece was planted. When the plants were about 3 to 5 inches high, virus-free aphids were intro-

[4] These aphids were identified by P. W. Mason, of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.

duced into the cage and permitted to feed back and forth on the diseased and healthy plants. This method should provide ideal conditions for the transmission of these diseases. By using as the source of infection tubers having leaf roll, which is readily transmitted by some species of aphids, in addition to a mosaic, evidence can be secured as to whether or not a selective method of transmission is practiced by any or all species of aphids.

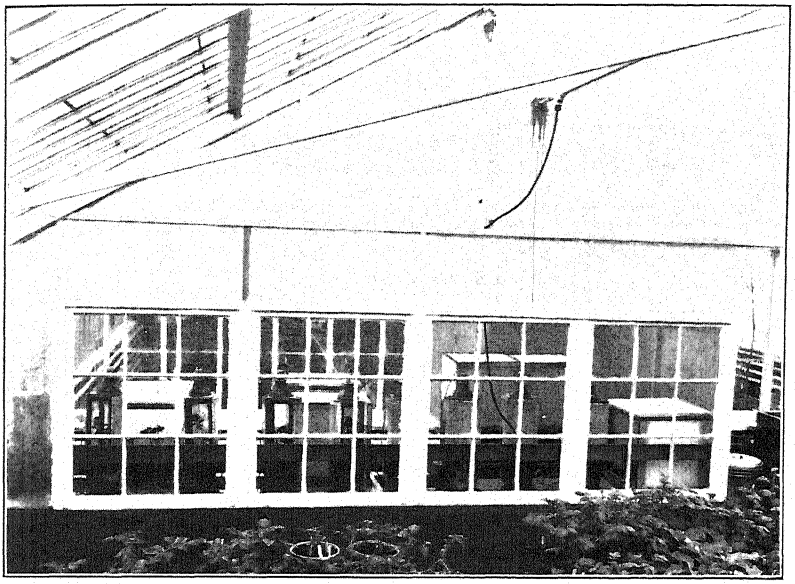


FIGURE 1.—Insectary in the Oregon Agricultural Experiment Station greenhouse, used to propagate different species of aphids on potato plants under individually covered cages. Rectangular cages were used to transfer aphids to tobacco and potato plants.

INSECTS OTHER THAN APHIDS

Eight large muslin-covered cages, each big enough to cover about 80 hills, were used to determine whether, in addition to aphids, those insects that naturally feed on potato vines are also able to transmit any of the potato viruses. A row of diseased plants infected with mild mosaic, crinkle mosaic, rugose mosaic, and leaf roll was planted in the center of the cage; on each side of this row were two rows of healthy potato plants, each row being planted with a different variety. The insects *Philaenus leucophthalmus*, *Lygus pratensis*, *Epitrix subcrinata*, *Nabis alternatus*, and *Empoasca filamenta* were collected from a field of healthy potatoes and deposited in a specially constructed small cage covered with black cloth except on one side, which had glass. This cage was placed in a field laboratory, with the glass facing the light. The insects fly toward the glass, where they congregate. By means of a sucking pipette, essentially like that used by Kunkel (*10*), it was a simple matter to pick up several individual insects belonging to one species. These were introduced into a large cage, each cage receiving a different species. Additional insects were added every

week, so that by midseason a population of several hundred insects in different stages of development was present in each cage. At digging time each hill of potatoes was harvested separately and planted the following spring to determine whether any of the different species of insects had transmitted any of the potato viruses.

DETERMINING THE FEEDING HABITS OF POTATO APHIDS

The following technique was used to study the feeding habits of potato aphids. Leaves and stems were cut from the plants on which aphids were colonized, with as little disturbance of the insects as possible. In 1934 these were dropped immediately into formalin acetic alcohol and left to fix for 24 hours, after which the usual alcohol series was used for dehydration. The embedding was carried out in the usual way by infiltration with xylol and paraffin, the material being placed in the 60° C. oven for 24 hours to allow evaporation of the xylol, after which it was run through several changes of paraffin and then embedded. It was found that many aphids had withdrawn their stylets and were washed off the tissues because of the slowness of the killing agent.

In 1935 and 1936 the aphids were killed instantly by dipping the leaves and stems bearing them into chloroform, and the material was then removed immediately and dropped into formalin acetic alcohol, where it was allowed to remain for 24 hours. The dioxane dehydration over calcium chloride was used. In using this process the tissues were de-aired in the fixative, removed from the fixative, washed in dioxane, and then placed in dioxane over calcium chloride for 24 hours. In 1935 the material was removed from the dioxane to a mixture made up of 4 parts dioxane, 1 part xylol, and 1 part paraffin, in which it was allowed to remain for an hour at room temperature. The material was transferred to a second mixture made up of 1 part dioxane, 1 part xylol, and 3 parts soft paraffin at 50° C. After an hour in this mixture the tissues were washed twice in soft paraffin and transferred to hard paraffin for embedding.

Since the method used in 1935 gave poor paraffin penetration, the procedure was modified somewhat the following year. After dehydration with dioxane, tissues were transferred to a series of mixtures of dioxane and xylol as follows:

1. Xylol 1 part, dioxane 4 parts.
2. Xylol 2 parts, dioxane 3 parts.
3. Xylol 3 parts, dioxane 2 parts.
4. Xylol 4 parts, dioxane 1 part.

The time in each mixture was from 10 to 15 minutes. Tissues were removed from mixture No. 4 to pure xylol, and paraffin embedding was carried out in the usual manner. This method gave excellent results with little disturbance of the aphids on the plant tissues

Materials were embedded in 56° C. paraffin. Experience has shown that to get good results in sectioning aphids the tissues must be placed in the paraffin in such a way that as many of the aphids as possible will lie with the long axis of the body at right angles to the cutting surface of the microtome knife and with the head toward the knife. It was found best to mark the paraffin blocks so that the orientation of the aphids within the paraffin was known.

Most of the sections were cut 20μ in thickness. The stylets range from about 10μ to over 15μ in diameter. When sections thinner than 20μ are cut many of the stylets are sectioned longitudinally For most purposes of study this is not desirable.

All sections cut were mounted serially and examined under the microscope. All records are based on observation of the stylets from the point where they entered the plant tissue to their tips. Where it appeared that the stylets had been partly withdrawn, or the entire stylets could not be found in serial sections, no record was taken. In many cases where the stylet had been withdrawn its path could be seen plainly, but records are based entirely on stylets observed in place in the tissues.

In all cases the first examination was made with the ribbon still in the paraffin. Those sections that did not show anything worth a thorough examination were discarded. The others were run up and stained in light green for more complete examination, and from the best of them permanent slides were made. Light green or fast green give better results when photographed than any other dyes that have been tried. In all, about 10,000 sections were cut and examined.

TRANSMISSION TESTS

TESTS WITH APHIDS

In 1929 *Myzus persicae*, *M. circumflexus*, and *M. solani* gave a very high percentage of infection when used to transmit leaf roll, but they almost always failed to transmit any of the other potato virus diseases. In only a few instances did *Macrosiphum* (*Illinoia*) *solanifolii* transmit leaf roll, and in no case did it transmit any of the other diseases.

In greenhouse experiments in the fall of 1932 and the spring of 1933 *Myzus persicae* and *Macrosiphum* (*Illinoia*) *solanifolii* were tested as vectors of the different virus diseases. Both sprout and plant feeding tests were conducted. As the source of infection, plants having both leaf roll and a mosaic disease were used. *Myzus persicae* consistently transferred leaf roll, in many cases 100-percent transmission being recorded, but only occasionally did it transfer the accompanying mosaic virus. Interveinal mosaic, crinkle mosaic, and rugose mosaic were transmitted by *M. persicae* but not by *Macrosiphum* (*I.*) *solanifolii*. This latter species also was considerably less effective as a vector of leaf roll, although occasionally a fairly high percentage of transmission was noted.

In 1933 experiments were conducted under cages in the field to test *Myzus persicae*, *M. solani*, and *Macrosiphum* (*Illinoia*) *solanifolii* as vectors of potato virus diseases. In order to avoid possible infection by root contact and to simulate natural conditions, a diseased tuber was planted in a 6-inch flowerpot and placed in the center of the cage. At each end of the cage was planted a healthy seed piece of the variety Bliss Triumph. When the plants were 3 to 5 inches high, virus-free aphids were introduced into the cage and permitted to feed on the diseased and on the healthy plants. This method was used in preference to the one that was previously employed, in which aphids were colonized on a diseased plant in a separate cage and then transferred to healthy plants. It was thought that by allowing the aphids to feed alternately on a diseased and a healthy plant the possibility might be eliminated of the virus being destroyed within the aphid before it had

a chance to feed on the healthy plant. The tubers of each exposed hill in the cage were harvested and planted in the spring of 1934, to determine the percentage of infection transmitted by the different species of aphids. The results showed (table 1) that a fairly high percentage of disease was transmitted by these insects.

TABLE 1.—*Results of aphid transmission of potato virus diseases in 1933 and 1934; work done in field cages, as manifested by performance of progeny in 1934 and 1935*

Aphid	Disease	Hill lots planted	Hill lots diseased	
		Number	*Number*	*Percent*
EXPERIMENT OF 1933-34				
Myzus persicae	Mild mosaic	43	33	77
Do	Crinkle mosaic	10	5	50
Do	Leaf-rolling mosaic	15	5	33
Do	Rugose mosaic	42	32	76
Do	Leaf-roll	20	16	80
M. solani	Mild mosaic	51	13	25
Do	Crinkle mosaic	24	3	12
Do	Rugose mosaic	27	1	4
Do	Leaf roll	18	18	100
Macrosiphum (Illinoia) solanifolii	Mild mosaic	14	4	28
Do	Rugose mosaic	22	9	41
Do	Leaf roll	30	6	20
EXPERIMENT OF 1934-35				
Macrosiphum (Illinoia) solanifolii	Mild mosaic	34	9	27
Do	Rugose mosaic	16	4	25
Do	Leaf roll	16	12	75
Myzus circumflexus	Mild mosaic	22	16	73

In 1934 the same method of transfer was used as in 1933 to test *Macrosiphum (Illinoia) solanifolii* and *Myzus circumflexus* as vectors of potato virus disease. The results are shown in table 1.

During the autumn and winter of 1934 an effort was made to determine the minimum number of aphids required and the length of time that the aphids must feed on diseased and healthy plants in order to transmit potato mosaic viruses. These studies were conducted in the greenhouse, and the same species of aphids were used as before, with the exception of *Myzus persicae*. The aphids were colonized on individually caged mosaic-infected potato plants, and a definite number of one species were transferred to young tobacco (*Nicotiana tabacum* L.) plants growing in pots. These plants were placed under a muslin-covered cage in an insectary in the greenhouse (fig. 1) and kept there for a week to 10 days, during which time the plants were examined two or three times and the aphids counted. In case fewer aphids than the original number were present, more were added. At the end of the feeding period the plants were placed in a fumigating box and the aphids were killed by Nicofume vapor. The tobacco plants were then kept in the greenhouse for observation.

The results were not consistent. Mild mosaic and crinkle mosaic were transmitted from potato to tobacco by *Myzus circumflexus*, *M. solani*, and *Macrosiphum (Illinoia) solanifolii*. In some series 20 to 100 percent of the plants became infected; in others, when the same species and the same number of aphids were left on the plants for the same length of time, infection did not occur. As few as five aphids

per plant have proved to be enough to cause infection, but often there was no infection even under what seemed to be the most favorable conditions from the standpoint of number of aphids used and the length of time they were permitted to feed. The most consistent infection was secured during the latter half of October and the first half of November in 1934.

Occasionally under greenhouse conditions a high percentage of mild and crinkle mosaic was transmitted from potato to tobacco, but at other times the results were entirely negative, although apparently the procedure was the same. This leads one to surmise whether such factors as light, humidity, or temperature, by affecting the physiology of the aphids may influence their capacity as vectors.

It was definitely demonstrated that both mild mosaic and crinkle mosaic can be transmitted from potato to tobacco by the three species of aphids tested. Although more than 300 tobacco plants were used in these studies, not once was the latent or X virus found in "commercial healthy" American potatoes transmitted by any of the aphids. These negative results are in agreement with those of other workers. Only the mild mosaic and the crinkle mosaic components free from the latent virus were transmitted. There was no observable consistent difference in the symptoms produced by these two viruses on tobacco plants. Instead of a vein banding, a mottling developed which resembled the mild mottling sometimes produced by the latent or X virus. It was found that these different viruses could be readily distinguished, however, by inoculating infected tobacco plants with tobacco mosaic. Tobacco plants infected with the X virus alone, upon the addition of tobacco mosaic, invariably develop a spot necrosis on the leaves, whereas the tobacco mosaic plants infected with the mild or crinkle mosaic virus free from the X virus develop only tobacco mosaic symptoms in addition to the mottling produced by the particular potato mosaic virus present.

The symptoms produced on tobacco by aphid-transmitted mild mosaic and crinkle mosaic correspond to the symptoms observed when the mild mosaic and crinkle mosaic were transmitted to tobacco (*5*) from U. S. D. A. seedling 41956, which is immune from the latent or X virus (*20*). In both cases the latent component is removed from the virus complex.

The aphid-transmitted crinkle mosaic and mild mosaic have been transferred from tobacco to Bliss Triumph and Green Mountain potato plants by juice transfers and also by stem grafting, the symptoms being typical of each disease in these varieties.

TESTS WITH INSECTS OTHER THAN APHIDS

In 1931, 180 small cages, each covering three potato plants, were used to test the ability of different species of insects, which occur naturally on potato, to act as vectors of potato virus diseases. Separate rows of potato plants infected with rugose mosaic, mild mosaic, and leaf roll were planted in the field. By means of an insect net, sweepings of these diseased plants were made, and the different species of insects, namely, the flea beetle (*Epitrix subcrinata*), the spittle bug (*Philaenus leucophthalmus*), the tarnished plant bug (*Lygus pratensis*), the leafhopper (*Empoasca filamenta*), and *Nabis alternatus*, collected from each diseased row, were segregated, and from 5 to 40 individuals

of each species were transferred to young plants from 4 to 6 inches high growing in cages. The tubers were saved from each caged hill to which insects had been admitted, and the next season they were planted as hill lots to determine the amount of disease, if any, that would be spread by the different species of insects. The results failed to show that any disease had been transmitted.

The same species of insects were tested again in 1933 and 1934. In these experiments eight large muslin-covered cages, each covering about 80 potato plants, were used. A row consisting of plants infected with one of the following diseases, namely, mild mosaic, crinkle mosaic, rugose mosaic, and leaf roll, was planted in the center of each cage. On each side of the center row were two rows of potatoes, each of a different variety. Insects were collected from healthy potato plants as previously described, and each cage received a different species. Additional insects were introduced each week, so that by the middle of the season several hundred insects belonging to one species were present in various stages of development in each cage. At harvest time each hill lot was saved and planted again as such the following spring. The results showed that these insects failed to transmit any of the diseases in 1933 and 1934.

In 1933 *Myzus persicae* was introduced into one cage when the plants were quite large, and the following spring the tubers from the exposed plants were planted. The results were as follows: In the variety Earliest of All, 58 plants out of 63 had leaf roll and 5 were healthy; of the 29 Bliss Triumph plants, 21 had leaf roll, 5 mild mosaic, and 3 rugose mosaic; of the 30 White Rose plants, 16 had leaf roll, 10 mild mosaic, and 4 rugose mosaic; and of the 23 Burbank plants, 21 had leaf roll and 2 were healthy. These results show that the conditions for transmitting the diseases were favorable. The fact that no transmission took place in the other large cages indicates that the insects tested other than aphids apparently are of minor if any importance in transmitting these potato virus diseases.

FEEDING HABITS OF APHIDS

A study of the feeding habits of the aphids showed that the species *Myzus persicae*, *M. solani* (fig. 2), and *M. circumflexus* (fig. 3), habitually feed in the phloem tissue (table 2). Only on rare occasions do they miss the vascular tissues, but no evidence was found that they feed in tissues outside the vascular bundles. In the few cases where the stylets had penetrated cells other than those of vascular tissues there was no evidence of plasmolysis of cells such as occurs when feeding takes place.

Thirty aphids of the species *Macrosiphum* (*Illinoia*) *solanifolii* (fig. 4), with stylets in place in the tissues, were studied. More than half of these were feeding in nonvascular tissue (fig. 5). Many were feeding too far from vascular tissue to be within reach of it. A considerable part of the nonvascular tissue in both the stem and leaves was plasmolyzed, indicating that the aphids actually were feeding there. All the aphids of this species observed were feeding on tender stem tips, possibly the feeding habits would be different on older stems.

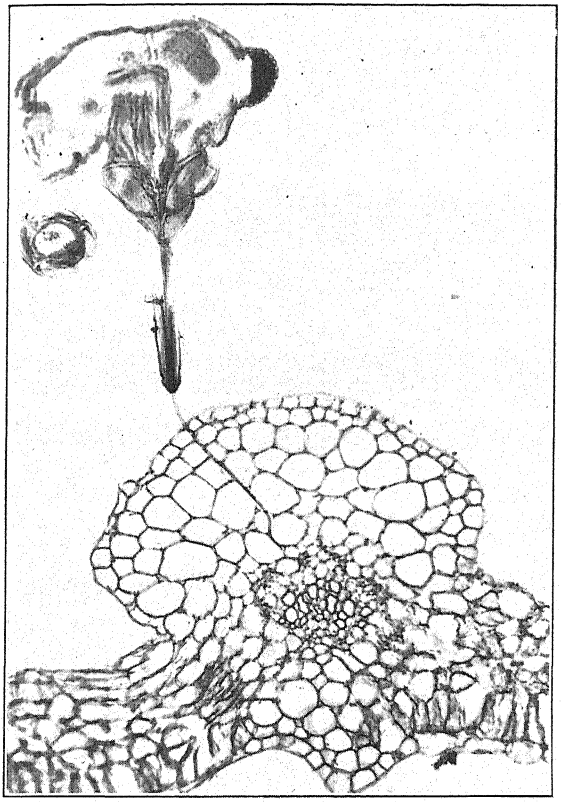


FIGURE 2.—Section of a potato leaf showing the path of the stylet of *Myzus solani*. Notice that the path was intercellular through the cortex, and that the aphid was feeding in the phloem tissue. × 80.

TABLE 2.—*Feeding methods of different species of aphids*

Species	Aphids observed	Aphids feeding in phloem of leaves	Aphids feeding in phloem of stems	Aphids feeding in mesophyll of leaves	Aphids feeding in nonvascular tissue of stem	Aphids feeding in vascular tissue	Aphids feeding in nonvascular tissue
	Number	*Number*	*Number*	*Number*	*Number*	*Percent*	*Percent*
Myzus persicae	45	44	0	1	0	97.8	2.2
M. circumflexus	27	12	14	1	0	96.3	3.7
M. solani	25	25	0	0	0	100.0	0
Macrosiphum (Illinoia) solanifolii	30	9	5	6	10	46.7	53.3

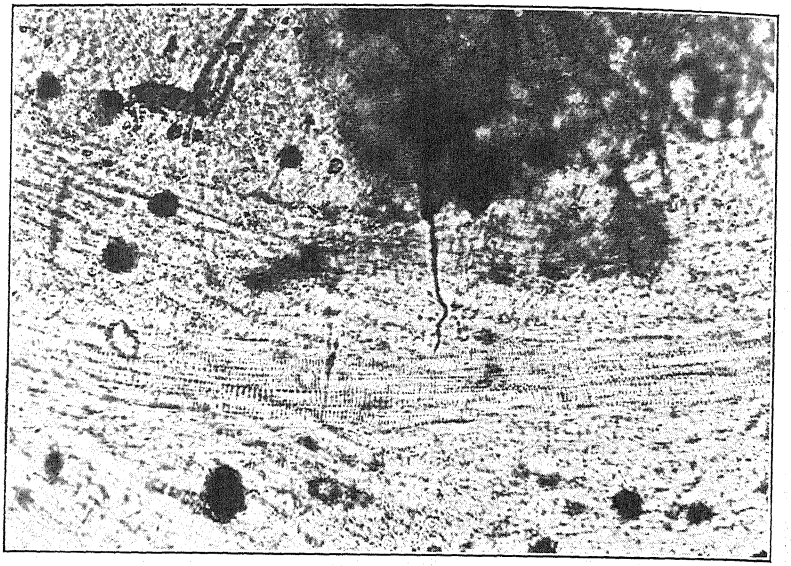


FIGURE 3.—Photograph from whole mount of leaf showing stylet of *Myzus circumflexus* within tissues of vascular area. × 80.

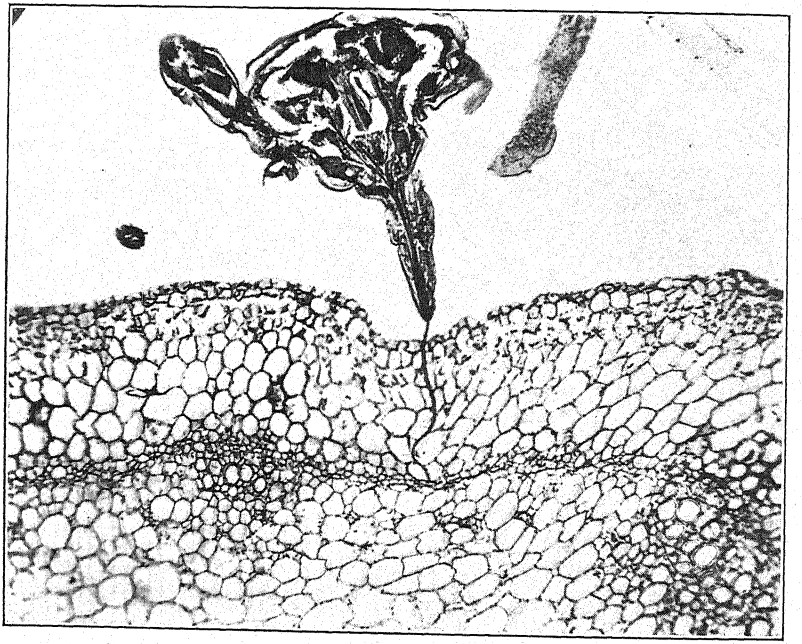


FIGURE 4.—Section of a potato stem showing *Macrosiphum (Illinoia) solanifolii* feeding in the vascular tissue. This type of feeding was observed in 47 percent of the cases noted. × 80.

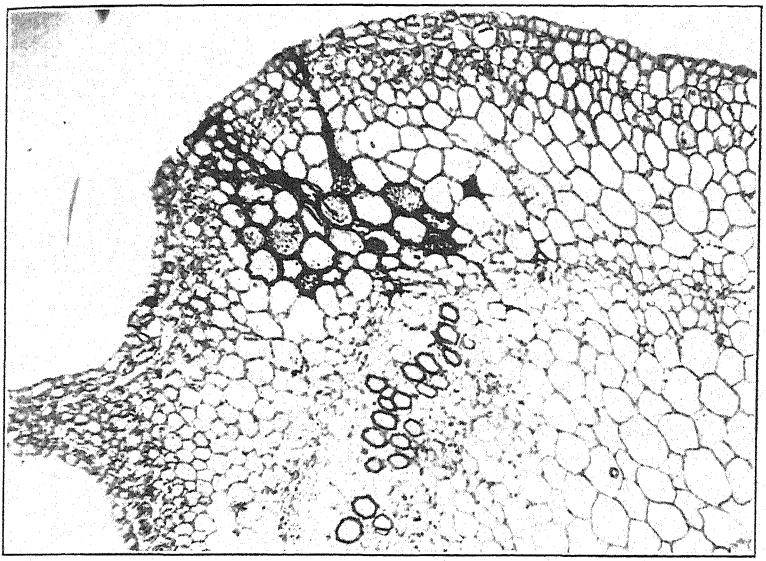


FIGURE 5.—Section of a potato stem showing the path followed by the stylet of *Macrosiphum (Illinoia) solanifolii*. Notice the plasmolysis of the cortex cells, indicating that the aphid was feeding in the cortex. This type of feeding was noted in 53 percent of the cases observed. × 80.

DISCUSSION AND CONCLUSIONS

The results secured show that all four species of aphids tested can serve as vectors of leaf roll, rugose mosaic, crinkle mosaic, and mild mosaic. *Myzus persicae*, *M. circumflexus*, and *M. solani* transmit leaf roll readily but often fail to transmit an accompanying mosaic when colonized on potato plants infected with a combination of leaf roll and one of the potato mosaics. The fact that in so many cases when aphids were taken from diseased to healthy plants leaf roll was transmitted to the exclusion of the mosaics indicates that the aphids fed on the source of infection as well as on the plants to be infected, and lack of transmission was apparently not due to the feeding habit.

Macrosiphum (Illinoia) solanifolii did not prove to be as effective a vector of leaf roll as the other three species of aphids studied, although occasionally a high percentage of transmission of this disease occurred. This discrepancy can possibly be explained by its feeding habits. *M. (I.) solanifolii* fed in the vascular tissues in only 46.7 percent of the cases observed, and it was the only one of the four species studied that fed less than 50 percent of the time in the vascular region. The others fed practically always in vascular tissue. Very little difference was observed in the ability of the three species of *Myzus* to transmit leaf roll. This evidence, although not conclusive, seems to indicate that phloem feeding is associated with the ability of aphids to transmit leaf roll.

The fact that all three *Myzus* species at times transmitted different potato viruses, whereas under apparently similar conditions they often failed to transmit the same viruses, suggests that the reason may be

physiological, and that perhaps such factors as light, temperature, or humidity may influence the virus within the aphid. Since in practically 100 percent of the cases observed these three species fed consistently in the phloem region, it appears that the variation in results cannot be explained on the basis of their feeding habits.

The fact that considerably more infection occurred when aphids had continuous access to the source of infection and the plants to be inoculated than when they were directly transferred from a diseased to a healthy plant suggests that the mosaic viruses may have a short longevity in the aphids.

Mild mosaic and crinkle mosaic were transmitted from potato to tobacco by *Myzus circumflexus*, *M. solani*, and *Macrosiphum (Illinoia) solanifolii*, although in many cases the results were entirely negative. As few as five aphids per plant have proved enough to cause infection.

Three years' testing of insects other than aphids, naturally feeding on potatoes, namely, the flea beetle (*Epitrix subcrinata*), the tarnished plant bug (*Lygus pratensis*), the leafhopper (*Empoasca filamenta*), the spittle bug (*Philaenus leucophthalmus*), and *Nabis alternatus* failed to show transmission of any of the potato viruses studied. Since the conditions under which these experiments were conducted were ideal for transmission, and since aphids transmitted these diseases readily under similar conditions, it would seem that these insects are unable to transmit leaf roll, rugose mosaic, crinkle mosaic, and mild mosaic.

SUMMARY

It has been demonstrated that four species of aphids, namely, *Myzus persicae*, *M. solani*, *M. circumflexus*, and *Macrosiphum (Illinoia) solanifolii*, under certain conditions are effective vectors of potato viruses. A much higher percentage of transmission of the potato mosaic viruses occurred when the aphids had continuous access to the source of infection and the plants to be inoculated than when they were transferred directly from a diseased to a healthy plant. This was not true of leaf roll, for by the latter method *Myzus persicae*, *M. solani*, and *M. circumflexus* generally caused a high percentage of transmission. *Macrosiphum (Illinoia) solanifolii* generally failed to transmit leaf roll by this method, although occasionally a fairly high percentage of the leaf roll virus was transmitted by this species.

Mild mosaic and crinkle mosaic free from the latent or X virus were transmitted from potato to tobacco by *Myzus circumflexus*, *M. solani*, and *Macrosiphum (Illinoia) solanifolii*.

Three years' testing of insects other than aphids, naturally feeding on potato plants, namely, the flea beetle (*Epitrix subcrinata*), the tarnished plant bug (*Lygus pratensis*), the leafhopper (*Empoasca filamenta*), the spittle bug (*Philaenus leucophthalmus*), and *Nabis alternatus*, failed to show any transmission of the potato viruses tested. The three *Myzus* species tested habitually fed in the phloem, whereas *Macrosiphum (Illinoia) solanifolii* fed in the vascular tissues in less than 50 percent of the cases observed.

LITERATURE CITED

(1) BÜSGEN, M.
1891. DER HONIGTAU. BIOLOGISCHE STUDIEN AN PFLANZEN UND PFLANZENLÄUSEN. Jena. Ztschr. Naturw. 25: 339–428.

(2) CLEVELAND, C. R.
1931. THE RELATION OF INSECTS TO THE TRANSMISSION OF POTATO LEAFROLL AND POTATO MOSAIC IN INDIANA. Ind. Agr. Expt. Sta. Bull. 351, 24 pp., illus.

(3) DAVIDSON, J.
1923. BIOLOGICAL STUDIES OF APHIS RUMICIS L. THE PENETRATION OF PLANT TISSUES AND THE SOURCE OF FOOD SUPPLY OF APHIDS. Ann. Appl. Biol. 10: 35–54.

(4) DYKSTRA, T. P.
1933. WEEDS AS POSSIBLE CARRIERS OF LEAF ROLL AND RUGOSE MOSAIC OF POTATO. Jour. Agr. Research 47: 17–32, illus.

(5) ———
1936. COMPARATIVE STUDIES OF SOME EUROPEAN AND AMERICAN POTATO VIROSES. Phytopathology 26: 597–606, illus.

(6) ELZE, D. L.
1927. DE VERSPREIDING VAN VIRUSZIEKTEN VEN DE AARDAPPEL (SOLANUM TUBEROSUM L.) DOOR INSECTEN. (TRANSMISSION EXPERIMENTS WITH INSECTS OF VIRUS DISEASES OF THE POTATO.) Meded. Landbouwhoogesch. Wageningen, deel 31, verhandel. 2, 90 pp., illus. [In Dutch. English summary, pp. 61–65.]

(7) FOLSOM, DONALD, SCHULTZ, E. S., and BONDE, REINER.
1926. POTATO DEGENERATION DISEASES: NATURAL SPREAD AND EFFECT UPON YIELD. Maine Agr. Expt. Sta. Bull. 331, pp. [59]–112.

(8) GARDNER, MAX W., and KENDRICK, J. B.
1928. POTATO MOSAIC AND LEAFROLL: SPREAD AND EFFECT ON YIELD. Ind. Hort. Soc. Trans. 67: 158–168.

(9) GOSS, R. W.
1929. THE RATE OF SPREAD OF POTATO VIRUS DISEASES IN WESTERN NEBRASKA. Jour. Agr. Research 39: 63–74, illus.

(10) KUNKEL, L. O.
1926. STUDIES ON ASTER YELLOWS. Amer. Jour. Bot. 13: 646–705, illus.

(11) MCKAY, M. B., and DYKSTRA, T. P.
1932. POTATO VIRUS DISEASES, OREGON INVESTIGATIONS 1924–1929. Oreg. Agr. Expt. Sta. Bull. 294, 40 pp., illus.

(12) MURPHY, PAUL A.
1924. INVESTIGATIONS ON THE LEAF-ROLL AND MOSAIC DISEASES OF THE POTATO. Ireland Dept. Agr. and Tech. Instr. Jour. 23: 20–34, illus.

(13) ——— and MCKAY, ROBERT.
1925. INVESTIGATIONS ON THE LEAFROLL AND MOSAIC DISEASES OF THE POTATO. Ireland Jour. Dept. Lands and Agr. 25: 138–154, illus.

(14) ——— and M'KAY, ROBERT.
1926. METHODS FOR INVESTIGATING THE VIRUS DISEASES OF THE POTATO, AND SOME RESULTS OBTAINED BY THEIR USE. Roy. Dublin Soc. Sci. Proc. 18: 169–184, illus.

(15) OORTWIJN BOTJES, JAN GERHARDUS.
1920. DE BLADROLZIEKTE VAN DE AARDAPPEL PLANT. 136 pp., illus. Wageningen. (Diss. Landbouwhoogesch. Wageningen.) [In Dutch. Referat in German, pp. [123]–136.]

(16) SCHULTZ, E. S., and FOLSOM, DONALD.
1921. LEAFROLL, NET-NECROSIS, AND SPINDLING-SPROUT OF THE IRISH POTATO. Jour. Agr. Research 21: 47–80, illus.

(17) ——— and FOLSOM, DONALD.
1923. TRANSMISSION, VARIATION, AND CONTROL OF CERTAIN DEGENERATION DISEASES OF IRISH POTATOES. Jour. Agr. Research 25: 43–118, illus.

(18) SCHULTZ, E. S., and FOLSOM, DONALD.
1925. INFECTION AND DISSEMINATION EXPERIMENTS WITH DEGENERATION DISEASES OF POTATOES. OBSERVATION IN 1923. Jour. Agr. Research 30: 493–528, illus.
(19) ——— FOLSOM, DONALD, HILDEBRANDT, F. MERRILL, and HAWKINS, LON A.
1919. INVESTIGATIONS ON THE MOSAIC DISEASE OF THE IRISH POTATO. Jour. Agr. Research 17: 247–274, illus.
(20) SCHULTZ, E, S., and RALEIGH, W. P.
1933. RESISTANCE OF POTATO TO LATENT MOSAIC. (Abstract) Phytopathology 23: 32.
(21) SMITH, KENNETH M.
1926. A COMPARATIVE STUDY OF THE FEEDING METHODS OF CERTAIN HEMIPTERA AND THE RESULTING EFFECT ON THE PLANT TISSUE, WITH SPECIAL REFERENCE TO THE POTATO PLANT. Ann. Appl. Biol. 13: 109–139, illus.
(22) ———
1927. OBSERVATIONS ON THE INSECT CARRIERS OF THE MOSAIC DISEASE OF POTATO. Ann. Appl. Biol. 14: 113–131, illus.
(23) ———
1929. STUDIES ON POTATO VIRUS DISEASES. V. INSECT TRANSMISSION OF POTATO LEAF-ROLL. Ann. Appl. Biol. 16: [209]–229, illus.
(24) ———
1931. STUDIES ON POTATO VIRUS DISEASES. IX. SOME FURTHER EXPERIMENTS ON THE INSECT TRANSMISSION OF POTATO LEAF-ROLL. Ann. Appl. Biol. 18: [141]–157, illus.

VERTICAL MIGRATION, DISTRIBUTION, AND SURVIVAL OF INFECTIVE HORSE STRONGYLE LARVAE DEVELOPING IN FECES BURIED IN DIFFERENT SOILS[1]

By John T. Lucker[2]

Associate zoologist, Zoological Division, Bureau of Animal Industry, United States Department of Agriculture

INTRODUCTION

Quantitative experiments on vertical migration of infective horse strongyle larvae in soils of different types have been reported recently by the writer (*6*).[3] The investigation reported in this paper was undertaken to determine: (1) Whether infective larvae develop from horse strongyle eggs in fresh feces buried in soils of different types; (2) the degree of vertical migration of the larvae in different types of soil; (3) the quantitative relationship between the number of eggs buried and the number of infective larvae reaching the surface of the soils at certain intervals; and (4) the distribution and total number of infective larvae present in the feces and soil at the expiration of these intervals. Obviously, the last three objectives could be realized only if development of infective larvae occurred in the buried feces. The work was carried out at the Agricultural Research Center, Beltsville, Md., from August 26, 1936, to March 22, 1937.

PROCEDURE AND APPARATUS

For this investigation, sandy clay loam, coarse sand, fine sandy loam, and clay soils were obtained from areas not occupied by horses. For sterilization, soil of each of these four types was separately placed in a lidded, metal-lined box provided at the bottom with a coil of perforated pipe connected to a steam boiler. Steam under a maximum boiler pressure of 25 pounds was admitted for 1 hour into the sterilizer containing the soil. Thermometers inserted in the soil at various points indicated that it was heated to temperatures of 180° to 212° F.; these temperatures are definitely known to be lethal to strongyle eggs and larvae. When examined by means of the Baermann apparatus, samples of soil so treated did not contain living nematode larvae.

Four containers were used for each type of soil. Each container consisted of a watertight, galvanized sheet-metal box 12 inches square and 4 inches deep and a piece of galvanized furnace pipe 10 inches in diameter. The length of the pipe in individual containers exceeded by about 5 inches the desired depth of burial of the feces in the soil subsequently placed in each container. In placing soil in a container, the box was first filled to a depth of about 3½ inches. The pipe was placed centrally on the soil in the box, and its lower edge was pressed down about one-half inch into the soil. The pipe was then filled with soil to within an inch of its upper edge.

[1] Received for publication, February 4, 1938; issued September, 1938.

[2] The writer is indebted to J. S. Andrews and A. G. Dinaburg, of the Zoological Division, for making the statistical analysis of the data in tables 2 and 3.

[3] Italic numbers in parentheses refer to Literature Cited, p. 347.

Freshly passed horse feces of two separate lots, collected on different dates, were used for this investigation. Each lot of feces was passed by a single horse. The fecal balls of each lot were thoroughly broken up and thoroughly mixed. Four-g samples from various parts of the fecal mass comprising each lot were taken for egg counts. The egg-count method described by Stoll (*13*) was used. Two 115-g portions of feces of each of the two lots were cultured in glass containers at room temperature for about a week. The cultures were then examined for infective larvae, and the average number recovered from the two cultures of each lot was noted.

An hour or two after their collection, the feces were buried to the desired depth in the soil in the various containers as follows: A weighed quantity of the feces was placed at the bottom of a cylindrical, flat-bottomed wire basket, 3 inches in diameter. The basket was made of galvanized-wire screen having about 64 meshes to the square inch. The depth of the basket was 3 inches greater than the depth to which it was desired to bury the feces. The quantity of feces used filled approximately the bottom 2¾ inches of the basket. During transfer of the feces to the basket, a paper insert prevented contact of the feces with the upper part of the basket. A circular piece of screen about 2⅞ inches in diameter was placed within the basket on the upper surface of the feces. A central excavation about 4 inches in diameter was made in the soil in the cylindrical part of the container to receive the wire basket (fig. 1). It was placed centrally down into the excavation, its upper edge extending slightly above the surface level of the soil in the pipe. The portion of the basket unoccupied by feces and the space around the basket were filled with soil previously removed from the excavation. This soil was gently pressed into place and leveled with the rest of the soil in the pipe. A container filled with soil and having the buried wire basket containing the feces in position is illustrated in figure 1.

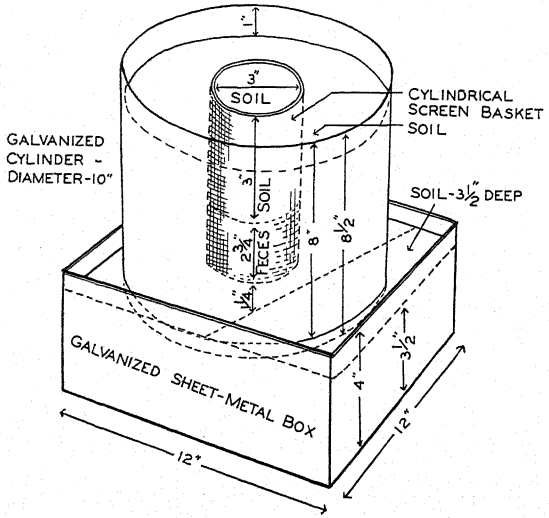


FIGURE 1.—Soil container used in this investigation; note position of wire basket containing feces. (The vertical dimensions of the cylinder and wire basket apply only to containers in which the feces were buried to a depth of about 3½ inches.)

Immediately after the soil was placed in the various containers it was thoroughly moistened with water. When the feces were buried a few days later, the soil was uniformly moist. Water was subsequently added to the surface of the soil in the pipe and in the box, in quantities and at intervals so regulated as to maintain the soil in a moist or damp condition at nearly all times throughout the investigation. On a few

occasions the surface layer of soil in various containers became dry. Whenever this was noted, water was promptly added. During the investigation the soil containers were kept in a room of a large building having southern and eastern exposures. From August 26, 1936, to October 15, 1936, outdoor temperatures in the vicinity of the building ranged from a maximum of 91° F. to a minimum of 39°; the mean temperature was 67°. During the remaining period of the investigation, the building was heated. Although the room used was not provided with a radiator, the temperature in it at all times remained above the freezing point. From October 28, 1936, to March 22, 1937, a maximum and minimum recording thermometer was kept in the room. Readings taken at frequent intervals indicated that the usual range of temperature during this time was from about 45° to 65°; a minimum of 38° and a maximum of 76° were recorded.

At various intervals after the feces were buried, a thin layer of soil about one-sixteenth to one-eighth inch in depth was scraped up with a spoon from the entire surface of the soil in the cylindrical part of each container. This soil was transferred to a Petri dish and examined for infective larvae soon after its collection. At intervals the soil thus removed was replaced by moist sterilized soil of the same type so as to maintain approximately the original surface level. Since the object of the examinations was to compare the degree of upward migration of larvae from the feces to the surface of the various soils, the surface of the soil in the box surrounding the pipe was not examined. Subsequent references to surface soil indicate soil from the pipe only.

At the expiration of desired intervals after burial of the feces, the removal of surface soil for examination was discontinued. Shortly thereafter, the contents of eight of the containers, two of each type of soil, were examined according to the following procedure:

A brass tube 1 inch in diameter and of suitable length was inserted in the soil in the pipe at a locus just alongside the outer edge of the wire basket and, hence, about 2 inches from the center of the soil. The tube was forced downward until the bottom of the box was reached. The soil removed when the tube was withdrawn was examined for larvae. A second column of soil was similarly obtained from a locus close to the edge of the pipe and, hence, about 4½ inches from the center of the soil; this soil was also examined for larvae. The soil was then removed from the wire basket down to the level of the wire disk resting on the feces, various levels of the soil being separately removed and separately examined for larvae. The wire basket was then readily withdrawn, and the feces were removed from the basket and examined for larvae. All soil remaining in the pipe was removed down to the level of the surface of the soil in the box. This soil was placed on a large sheet of heavy paper and thoroughly pulverized and mixed. It was then rolled about on the paper for at least 30 minutes in the way that ores are sometimes mixed for sampling. Two samples of the soil, each 16 cubic inches in volume, were removed and examined for larvae. From the average number of larvae recovered in these two samples, the total larval content of the known volume of soil removed from the pipe was computed. The empty pipe was lifted from the soil in the box. By means of the brass tube, a column of soil was removed from the center of the box and a second sample was taken from one corner of the box; these were separately

examined for larvae. The soil remaining in the box was removed and subjected to the process of mixing, sampling, and examination, as previously noted. The approximate number of larvae in this soil was computed.

The object of removing, by means of the brass tube, vertical columns of soil from the specified locations was to permit comparison of the number of larvae in equal volumes of soil near the feces and horizontally distant from it. Owing to compaction of the soil as the tube was forced downward and to other factors, the height of the column of soil removed by the tube was always less than the actual depth of soil in the containers. The quantity removed was also influenced by the type of soil, but in a given type, approximately the same quantity was withdrawn by the tube after insertion at comparable loci. Hence, comparisons of the number of larvae recovered in these approximately equal volumes of soil from different horizontal loci gave an index of the degree of lateral migration from the feces in a particular soil.

Examinations of the soil and feces for larvae were made by means of the Baermann isolation apparatus. The depth of soil placed on the screen of an individual Baermann apparatus did not exceed one-half inch. A piece of muslin or silk bolting cloth was placed on the screen to prevent descent of large particles of soil. The soil or feces remained in the Baermann apparatus a minimum of 48 hours before fluid was withdrawn from the bottom of the rubber tube. Successive quantities of the fluid, each about 10 cc in volume, were withdrawn into Syracuse watch glasses. As a rule, these successive withdrawals were continued until the fluid in the last one or two watch glasses examined contained no larvae. Occasionally, when relatively large numbers of larvae were present in the fluid, withdrawals were discontinued when the few larvae found in the contents of the last few watch-glass samples examined were a negligible percentage of the total number of larvae previously recovered. On recoveries of up to about 1,000 larvae, counts were direct. A ruled watch glass or a Scott counting slide was used in making these counts. When larger numbers of larvae were recovered, counts were made by means of a dilution method.

EXPERIMENTAL DATA

STRONGYLE EGG CONTENT OF THE FECES BURIED AND PERCENTAGE OF DEVELOPMENT IN CULTURES

Data on the strongyle egg content and the percentage of development in cultures of the two lots of horse feces used in this investigation are given in table 1. On the respective dates of collection, as shown in this table, 115 g of feces of lot 1 was buried in the soil in each of the four containers of sandy clay loam, and 115 g of feces of lot 2 was buried in the soil in each of the four containers of fine sandy loam, coarse sand, and clay.

As shown by the data of table 1, the strongyle egg content of 115 g of feces of lot 1 was almost twice that of the same quantity of feces of lot 2. No important difference in ability of the eggs to develop and produce infective larvae was indicated by the average percentage of development obtained in the cultures of feces of the two lots.

TABLE 1.—*Strongyle egg content of each of 2 lots of feces and percentage of development in cultures*

Lot No.	Date feces were collected	Egg counts	Eggs per gram of feces (average of all counts)	Eggs in 115 g of feces	Infective larvae from cultures of 115 g of feces (average of 2 cultures)	Development of eggs to infective larvae in cultures (average of 2 cultures)
		Number	*Number*	*Number*	*Number*	*Percent*
1	Aug. 26, 1936	4	1,802	207,230	77,140	37.2
2	Sept. 4, 1936	11	933	107,300	43,800	40.8

RECOVERY OF INFECTIVE LARVAE IN THE SURFACE LAYER OF SOILS

The data on recovery of infective larvae in the surface layer of the four types of soil (tables 2 and 3) show that infective larvae developed from strongyle eggs contained in horse feces buried 3½, 6½, 8½, and 10½ inches in fine sandy loam and coarse sand, and 3, 6, and 8 inches in sandy clay loam, but afford no evidence as to development in feces buried in clay soil, since no larvae were recovered in the series of the examinations of the surface layer of this soil. On the sixth day after burial of the feces to depths of 3 to 3½ inches, some larvae reached the surface of sandy clay loam, coarse sand, and fine sandy loam. The interval between the burial of the feces and the first recovery of infective larvae in the surface layer of these three types of soil tended to increase with increased depth of burial. An inverse relationship between the total number of larvae recovered from the surface of each of the three types of soil, and the depth of burial of the feces is shown by the data.

The rate of migration of infective larvae to the surface of sandy clay loam, coarse sand, and fine sandy loam, after burial of the feces to any of the four depths tested, showed marked irregularity. The rate tended to reach a maximum within about 6 weeks after burial of the feces and to decline irregularly thereafter.

Comparison of the total number of larvae reaching the surface layer of the different soils in about 3½ to 5½ months shows that the greatest number of larvae reached the surface of fine sandy loam soil; this was true for each of the four depths tested. The number of larvae recovered from the surface layer of the other soils during a similar period were of decreasing magnitude, according to the following sequence: Coarse sand, sandy clay loam, and clay. This sequence was applicable to any of the four depths of burial. In the interval mentioned, the surface of fine sandy loam and coarse sand yielded considerable numbers of larvae. The maximum number, equivalent to 8.9 percent of the buried eggs, was from the surface of fine sandy loam after burial of the feces 3½ inches. However, when the depth of burial of feces was 8½ or 10½ inches in either fine sandy loam or coarse sand, the larvae recovered from the surface during the period of observation represented less than 1 percent of the eggs buried. Insignificant numbers of larvae, representing negligible percentages of the eggs buried, reached the surface of sandy clay loam in 101 to 171 days after burial of the feces 3 to 8 inches.

TABLE 2.—*Infective larvae recovered in series of examinations of the surface layer of three types of soil following burial of fresh horse feces containing 107,300 strongyle eggs*

Soil type	Distance from surface of feces to surface of soil	Infective larvae recovered in surface soil removed at indicated number of days after burial of feces																			Total infective larvae recovered	Larvae recovered on surface in relation to eggs buried
		5	6	8	11	14	17	26	33	41	47	54	61	68	75	83	90	104	126	162		
	Inches	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
Fine sandy loam	3½	0	9	115	107	829	303	1,561	350	1,315	1,065	781	806	689	137	374	224	628	269	(1)	9,562	8.9
	6½	0	0	10	11	112	43	68	458	747	275	245	582	311	62	239	82	242	165	46	3,698	3.5
	8½	0	0	1	1	65	28	76	240	139	71	34	50	38	1	36	0	89	46	5	920	0.9
	10½	0	0	0	0	0	4	2	28	7	9	2	15	5	1	16	3	16	4	2	114	.1
Coarse sand	3½	0	7	37	17	537	106	458	305	157	121	75	155	115	339	130	37	165	9	(1)	2,770	2.6
	6½	0	0	2	3	72	69	106	76	25	28	19	70	38	10	6	1	15	4	1	545	.5
	8½	0	0	0	0	64	52	100	100	49	24	23	25	17	9	8	1	21	2	1	496	.5
	10½	0	0	0	0	7	6	11	11	1	0	0	2	1	0	0	0	0	1	0	40	.04
Clay	3½	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	------	0	(1)	0	0
	6½	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	------	0	0	0	0
	8½	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	------	0	0	0	0
	10½	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	------	0	0	0	0

[1] As subsequently indicated, the soil was removed from these containers and examined previous to this time.

TABLE 3.—*Infective larvae recovered in a series of examinations of the surface layer of sandy clay loam following burial of fresh horse feces containing 207,230 strongyle eggs*

Distance from surface of feces to surface of soil (inches)	Infective larvae recovered in surface soil removed at indicated number of days after burial of feces																			Total infective larvae recovered	Larvae recovered on surface in relation to eggs buried
	5	6	8	10	13	16	23	31	38	45	52	59	66	75	80	87	94	101	140	171	
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
3	0	29	18	3	3	24	11	19	11	19	4	5	1	0	0	3	2	0	(1)	(1)	152	0.07
6	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	2	.0009
8	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	2	.0009
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[1] As subsequently indicated, this soil was removed from the container and examined on the one hundred and tenth day after burial of the feces.

Statistical analysis of the data in tables 2 and 3, made by Fisher's method (*5*), showed that depth of burial independent of soil type, and soil type independent of depth of burial, had a greater effect on number of larvae recovered than did sampling and random variation. No significant difference was found between the effects of soil type and depth of burial.

EFFECT OF SOIL TYPE AND DEPTH OF BURIAL ON DISTRIBUTION AND NUMBER OF LARVAE

The data on the distribution and number of larvae in the feces and soil in eight of the containers (table 4) show that many infective larvae developed from eggs in feces buried 3½ and 8½ inches [4] in clay soil. Development of larvae in feces buried in this soil was not evident from the data presented in table 2. Table 4 also shows that about 3½ to 6 months after the feces had been buried 3½ or 8½ inches in clay or 3 or 8 inches in sandy clay loam, comparatively large proportions of the larvae recovered occurred in the feces and in the soil immediately above the feces. In coarse sand and fine sandy loam, however, there was somewhat more uniform distribution of larvae with respect to the feces and the soil at all levels directly above it. This tendency toward uniformity of vertical distribution of the larvae was most marked in fine sandy loam after burial of the feces to a depth of 3½ inches. These facts are in harmony with the comparative degree of migration to the surface layer previously shown to occur in the respective soils from the respective depths (3 and 8 inches in sandy clay loam; 3½ and 8½ inches in the other soils).

TABLE 4.—*Number and distribution of infective larvae in feces and soil in 8 containers*

Soil type	Distance from surface of feces to surface of soil [1]	Interval from burial of feces to date of examination of soil and feces	Larvae recovered in columns of soil removed at indicated horizontal loci				Larvae in soil removed from indicated levels within wire basket							Larvae recovered from feces	Larvae [2] in remainder of soil in—		Total larvae recovered
			2 inches from center of cylinder	4½ inches from center of cylinder	Center of box	Corner of box	Surface to 1-inch level	1- to 2-inch level	2-inch level to surface of feces	Surface to 2-inch level	2- to 4-inch level	4- to 6-inch level	6-inch level to surface of feces		Cylinder	Box	
	In.	*Days*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
Sandy clay loam	3	110	465	0	64	0	31	181	13,729	----	----	----	----	8,200	38,534	595	61,799
	8¼	173	413	1	191	0	----	----	----	0	0	8	4,500	6,575	16,850	1,458	29,996
Fine sandy loam	3¾	126	178	0	2	0	411	275	624	----	----	----	----	442	1,984	68	3,984
	8½	173	68	56	15	0	----	----	----	39	266	248	1,045	453	5,030	365	7,585
Coarse sand	3½	146	49	1	4	0	40	51	112	----	----	----	----	976	1,094	54	2,381
	8	180	69	5	0	0	----	----	----	3	20	23	91	216	436	0	863
Clay	3¾	152	5	0	0	0	0	0	6,035	----	----	----	----	4,120	1,874	14	12,048
	8½	187	53	0	6	0	----	----	----	0	0	17	143	1,436	1,524	248	3,427

[1] The distances shown were measured when the soil was excavated from the wire baskets and in most cases differ slightly from those given in tables 2 and 3, which were the original depths of burial. These differences were caused by settling of the soil and compaction of the feces.

[2] Computed from number of larvae recovered in samples.

[4] These and other depths of burial subsequently mentioned are the original depths of burial; as indicated in table 4, unimportant slight variations from these depths were noted when the soil was excavated from the containers.

Data on larvae recovered from two horizontal loci in the cylindrical part of the containers (table 4) show that in about 3½ to 6 months after burial of the feces to depths of 3 to 3½ and 8 to 8½ inches in the four types of soil, larvae were mainly concentrated in the soil horizontally near the location of the feces. However, in fine sandy loam, after burial of the feces to a depth of 8½ inches, a considerable degree of horizontal migration of the larvae occurred. The data suggest that the degree of horizontal migration was affected by the type of soil in which the larvae were present and that in a given soil it tended to increase with increased depth of burial.

The recovery of infective larvae in the soil in the eight boxes indicates a general tendency for a small proportion of the larvae developing in the feces to pass downward in the soil. Except in coarse sand, the proportion of larvae which migrated downward was larger when the feces were buried to the greater of the two depths. The larvae were evidently located mainly in the central part of the box since none were found in the soil removed from one corner of each box.

That the total number of larvae remaining in the fecal and soil content of the eight containers 3½ to 6 months after burial of the feces was influenced by the type of soil in which burial occurred is indicated by the data in table 4. Most striking are the comparatively small numbers of larvae recovered in coarse sand and the comparatively large numbers recovered in sandy clay loam. In each of the soils, except fine sandy loam, the number of larvae recovered after burial of the feces at a depth of 3 to 3½ inches was considerably greater than the number recovered when burial was at a depth of 8 to 8½ inches. An influence of depth of burial on development or survival, or both, is indicated. However, from 34 to 63 days intervened between examinations of the contents of the series of containers in which burial of the feces was 3 to 3½ inches and the series in which it was 8 to 8½ inches. It is possible, therefore, that the results may have been influenced to some degree by death of larvae during these intervals.

RELATION BETWEEN NUMBER OF INFECTIVE LARVAE RECOVERED AND NUMBER OF EGGS BURIED

Table 5 shows the relation between the total number of larvae recovered from two containers of each of the four types of soil and the total number of strongyle eggs in the feces buried in these containers.

The percentage relationship between total number of infective larvae recovered and eggs buried was greatest in sandy clay loam for both depths of burial. The total number of larvae recovered after burial of the feces 3 inches in this soil closely approached the average number obtained in two control cultures of feces of lot 1. For the other three soils, the percentage of eggs recovered as infective larvae decreased in magnitude according to the following sequence: Fine sandy loam, clay, and coarse sand. This sequence applies to both depths of burial. In all four types of soil, the total number of larvae recovered in relation to eggs buried was greater when burial of the feces was at the shallower of the two depths. This is in agreement with the statement previously made concerning the probability that increased depth of burial caused an increase in deleterious effect. Table 5 also shows that in clay or sandy clay loam the migration of infective larvae to the surface after burial of the feces at a depth of 3 to 3½ or 8 to 8½ inches was either entirely prevented or greatly

inhibited. However, in fine sandy loam or coarse sand, a majority of all the larvae recovered in the indicated intervals after burial of the feces at a depth of 3½ inches had reached the surface layer.

TABLE 5.—*Relation between number of larvae recovered from 8 containers and number of eggs originally buried*

Soil type	Eggs buried	Distance from surface of feces to surface of soil [1]	Interval from burial of feces to final examination of surface soil	Larvae recovered from surface soil		Interval from burial of feces to removal and examination of feces and soil from containers	Larvae recovered from feces and soil upon removal from containers		Total larvae recovered	Proportion of larvae recovered to eggs buried
	Number	*Inches*	*Days*	*Number* [2]	*Percent* [3]	*Days*	*Number* [4]	*Percent* [3]	*Number*	*Percent*
Sandy clay loam	207, 230	3	101	152	0.25	110	61, 799	99.75	61, 951	29.9
	207, 230	8¼	171	2	.007	173	29, 996	99.99	29, 998	14.5
Fine sandy loam	107, 300	3¾	126	9, 562	70.6	126	3, 984	29.4	13, 546	12.6
	107, 300	8½	162	920	10.8	173	7, 585	89.2	8, 505	7.9
Coarse sand	107, 300	3½	126	2, 770	53.8	146	2, 381	46.2	5, 151	4.8
	107, 300	8	162	496	36.5	180	863	63.5	1, 359	1.3
Clay	107, 300	3¾	126	0	0	152	12, 048	100.0	12, 048	11.2
	107, 300	8½	162	0	0	187	3, 427	100.0	3, 427	3.2

[1] See footnote 1 of table 4.
[2] As shown in tables 2 and 3.
[3] Represents the percentage of the total number of larvae recovered.
[4] As shown in table 4.

DISCUSSION

Ackert (*1*) and Payne (*9*) have reported that eggs of the human hookworm (*Necator americanus*) hatched in feces buried to various depths beneath different soils and that there was subsequent migration of infective larvae to the soil surface. So far as has been determined by a review of the literature, the only similar study dealing with the effects of burial of feces in soil on the development of eggs of strongyle parasites of domestic animals has been reported by Spindler (*12*). He found that when eggs of *Oesophagostomum dentatum*, the common nodular worm of swine, were buried outdoors at various depths beneath the surface of sandy clay soil, they hatched and the larvae migrated to the surface.

That burial of feces containing horse strongyle eggs in soils likewise did not prevent development of infective larvae in the feces is amply shown by the data of the present paper. The data pertaining to vertical migration of infective horse strongyle larvae in the soils used in this investigation and the results of the writer's (*6*) earlier experiments, in which larvae already in the infective stage were buried in similar soils, agree in indicating a lack of migration to the surface in clay soil and an inverse relationship between depth of burial and number of larvae reaching the surface in other soils. A detailed comparison of the mathematical results of the two investigations is impracticable for obvious reasons. However, both investigations show that lighter soils, such as fine sandy loam, are most favorable for vertical migration of horse strongyle larvae.

The quantitative data supplied by this investigation are in part based on methods and procedures generally used in experimental

helminthology. That these and other methods of this investigation, adopted as the most suitable available, do not yield wholly accurate results is recognized. Since the procedures used were carried out in as uniform a manner as possible, the mathematical values obtained are regarded as satisfactory for comparison of the effect of burial at the different depths in the different types of soil on the eggs and resultant larvae. The purpose of the quantitative procedure used was to provide an approximate basis for conclusions for which qualitative data would afford no basis.

The experimental results were obtained under intentionally restricted conditions as to variation in temperature and moisture.

Temperatures in the room where the soils were kept were never so low as freezing, nor excessively high. It appears that these conditions of temperature were more favorable for development of the eggs and migration of the larvae than might frequently be encountered under field conditions. Whether these conditions of temperature were favorable or unfavorable for survival of the larvae is less evident. Augustine (*3*) has studied the influence of temperature on the life span of infective larvae of the human hookworm (*Necator americanus*). Larvae on soil were exposed to temperatures of 0°, 16°, 20° to 31°, 35°, and 40° C.; the larvae kept at 16° survived longest. Payne (*10*) found that the activity of infective larvae of this species increased with increased temperatures up to 35° C. According to Augustine (*2*), environmental conditions, such as tropical temperatures which tend to increase the activity of mature hookworm larvae, will shorten their lives by the more rapid using up of the stored food material. The abundance of food granules has been considered by Payne (*10*) to be an index to the "physiological age" of the larvae. Some other investigators have reported that infective larvae of human hookworms survive longest at room temperature. Horse strongyle larvae are known to be unusually resistant to low temperatures. Alternate freezing and thawing has been found by Ober-Blöbaum (*7*) to be more quickly lethal to these larvae than continuous freezing. The range of temperatures optimum for the survival of infective horse strongyle larvae has not been experimentally determined, so far as the writer is aware.

During this investigation, the soils were kept moist at practically all times, a condition favorable for development of the eggs and larvae and doubtless more conducive to the migration of the infective larvae than would occur ordinarily in a similar period in the field. Whether such a more or less uniform moisture content of the soil was favorable to the survival of the larvae is difficult to determine from such experimental evidence as is available. Augustine (*3*) found the length of life of infective larvae of *Necator americanus* to be longer in moist soils than in water-covered soils, drying soils, or soils subjected to alternate drying and moistening. Payne (*8*) also found a high death rate of human hookworm larvae in waterlogged soils. So far as the writer is aware, the effect of moisture content on the length of life of horse strongyle larvae in soils has not been studied. These larvae may live at least 6 to 8 months in water, according to the reports of various investigators summarized by Enigk (*4*). Horse strongyle larvae, moreover, are very resistant to drying. A fraction of 1 percent of more than 1,000 larvae kept in a dry condition on a glass surface for 4¼ years were viable, according to Enigk (*4*). Raffensperger (*11*) reported that all larvae exposed to drying in an incubator at

24°–26° C. for 6 months were dead; about 10 percent survived exposure for 4 months and 2 days. Alternate drying and wetting reduces the survival of the larvae, according to Ober-Blöbaum (*7*). Taylor (*14*) states that stability of moisture content in the loose soil of tilled land favors the survival of larvae of sheep nematodes as compared with survival under conditions of rapid alternation of moisture and dryness. In the paper referred to, Taylor does not give the experimental basis for this statement.

It thus seems likely that, in this investigation, temperature and moisture were favorable for the development of eggs and larvae and were more favorable for migration of infective larvae than would ordinarily be the case under outdoor conditions. It is less likely that the experimental temperatures and degree of moisture were particularly favorable to survival of the larvae. Conditions similarly affecting survival of the larvae would occur in regions having relatively warm fall and winter seasons with frequent rainfall.

However, general interpretation of the results of this investigation in relation to the control of strongyle parasitism in horses would involve a priori judgments as to the probable effect of many factors not operative in the experiments but capable under field conditions of producing results differing from those obtained in the laboratory. From this investigation, and from another reported earlier by the writer (*6*), fundamental information on the behavior of buried horse strongyle eggs and larvae has been obtained, but the extent to which the laboratory results may be altered under actual field conditions can be determined only by experimental investigation under such conditions. Until such field tests have been completed and the practicability of the measure ascertained, plowing under of infested soils is not advocated for the control of horse strongyles.

SUMMARY AND CONCLUSIONS

Burial of freshly passed horse feces containing strongyle eggs in four types of soil did not prevent development of infective larvae in the feces.

Insignificant numbers of infective larvae, representing 0.0009 to 0.07 percent of the eggs buried, reached the surface of sandy clay loam in 101 to 171 days after burial of the feces to depths of 3, 6, and 8 inches. No infective larvae had reached the surface of this soil 171 days after burial of the feces to a depth of 10 inches or the surface of clay soil in 126 to 162 days after burial of the feces 3½ to 10½ inches. However, the deeper soil layers and the feces still constituted important sources of infective larvae about 4 to 6 months after burial of the feces to depths of 3 to 3½ and 8 to 8½ inches in these soils.

Infective larvae reaching the surface in 126 to 162 days after burial of the feces 3½ to 10½ inches in fine sandy loam and coarse sand represented 0.04 to 8.9 percent of the eggs buried. Less than 1 percent of the eggs buried were represented by infective larvae reaching the surface of these soils from depths of 8½ and 10½ inches. Fine sandy loam was the most favorable soil for vertical migration of the infective larvae. Some infective larvae reached the surface of fine sandy loam, coarse sand, and sandy clay loam on the sixth day after burial of the feces to a depth of 3 or 3½ inches. The ratio of infective

larvae reaching the surface to eggs buried was inversely related to depth of burial in these three types of soil.

A considerable proportion of the larvae remained in the feces and the soil immediately above it 110 to 187 days after burial of the feces to depths of 3 to 3½ and 8 to 8½ inches in clay and sandy clay loam. More uniform distribution of the larvae with respect to the feces and the soil at various levels above it occurred in fine sandy loam and coarse sand. The degree of horizontal migration of larvae from the buried feces was, in general, not extensive; it apparently was affected by soil type and depth of burial. A general tendency for a small proportion of the larvae to pass downward in the various soils was shown. In three of the soils, this proportion was larger after burial of the feces to the greater of the two depths.

The total number of infective larvae reaching the surface in 101 to 152 days and the residuum of larvae in the feces and the soil 110 to 152 days after burial of feces 3 to 3½ inches in soils of the four types, accounted for 4.8 to 29.9 percent of the eggs buried. Of the eggs buried, 1.3 to 14.5 percent were represented by infective larvae reaching the surface in 162 to 171 days and remaining in the feces and soil 173 to 187 days after burial in the four types of soil to depths of 8 to 8½ inches. These percentages were highest for both depths when burial was in sandy clay loam, intermediate in clay or fine sandy loam, and lowest in coarse sand.

Statistical analysis of the data on larvae recovered from the surface of the soils showed that migration to the surface was significantly affected by soil type. This factor also appeared to influence the numbers of larvae persisting in the feces and deeper soil layers a few months after the feces were buried. Increased depth of burial in a given soil clearly reduced the degree of migration of infective larvae from the feces to the soil surface. Statistical analysis also showed that the effect of depth of burial, independent of soil type, was significant. Depth of burial also appeared to be a factor in determining the development or persistence of larvae in the feces and deeper soil layers. It is probable that the inverse relationship between depth of burial and amount of migration of larvae to the surface is in some degree related to the effect of depth of burial on larval development, or survival, or both. Deleterious effect on development of larvae and on survival of larvae cannot be differentiated by the data of this investigation.

LITERATURE CITED

(1) ACKERT, JAMES E.
1923. INVESTIGATIONS ON THE CONTROL OF HOOKWORM DISEASE. XIII. OBSERVATIONS ON CONDITIONS UNDER WHICH HOOKWORM EGGS AND LARVAE DEVELOP. Amer. Jour. Hyg. 3: 26–34.

(2) AUGUSTINE, DONALD L.
1922. INVESTIGATIONS ON THE CONTROL OF HOOKWORM DISEASE. X. EXPERIMENTS ON THE LENGTH OF LIFE OF INFECTIVE HOOKWORM LARVAE IN SOILS. Amer. Jour. Hyg. 2: 177–187.

(3) ———
1923. INVESTIGATIONS ON THE CONTROL OF HOOKWORM DISEASE. XXIII. EXPERIMENTS ON THE FACTORS DETERMINING THE LENGTH OF LIFE OF INFECTIVE HOOKWORM LARVAE. Amer. Jour. Hyg. 3: 420–443, illus.

(4) ENIGK, KARL.
1934. DIE WIDERSTANDSFÄHIGKEIT DER ENTWICKLUNGSSTADIEN DER STRONGYLIDEN AUSSERHALB DES WIRTSTIERES. Arch. Wiss. u. Prakt. Tierheilk. 67: [363]–376.

(5) FISHER, RONALD AYLMER.
1934. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 5, rev. and enl., 319 pp., illus. Edinburgh and London.
(6) LUCKER, J. T.
1936. EXTENT OF VERTICAL MIGRATION OF HORSE STRONGYLE LARVAE IN SOILS OF DIFFERENT TYPES. Jour. Agr. Research 52: 353–361.
(7) OBER-BLÖBAUM, W.
1932. UNTERSUCHUNGEN ÜBER DIE EINWIRKUNGEN PHYSIKALISCHER EINFLÜSSE AUF DIE LARVEN VON PFERDESTRONGYLIDEN. Tierärztl. Rundschau 38: 812–815.
(8) PAYNE, FLORENCE KING.
1922. INVESTIGATIONS ON THE CONTROL OF HOOKWORM DISEASE. XI. VERTICAL MIGRATION OF INFECTIVE HOOKWORM LARVAE IN THE SOIL. (PRELIMINARY REPORT.) Amer. Jour. Hyg. 2: 254–263, illus.
(9) ———
1923. INVESTIGATIONS ON THE CONTROL OF HOOKWORM DISEASE. XIV. FIELD EXPERIMENTS ON VERTICAL MIGRATION OF HOOKWORM LARVAE. (PRELIMINARY REPORT.) Amer. Jour. Hyg. 3: 46–58.
(10) ———
1923. INVESTIGATIONS ON THE CONTROL OF HOOKWORM DISEASE. XXX. STUDIES ON FACTORS INVOLVED IN MIGRATION OF HOOKWORM LARVAE IN SOIL. Amer. Jour. Hyg. 3: 547–583, illus.
(11) RAFFENSPERGER, H. B.
1931. INTERNAL PARASITES OF THE HORSE. Vet. Alumni Quart. [Ohio State Univ.] 18: 171–179.
(12) SPINDLER, L. A.
1936. EFFECTS OF VARIOUS PHYSICAL FACTORS ON THE SURVIVAL OF EGGS AND INFECTIVE LARVAE OF THE SWINE NODULAR WORM, OESOPHAGOSTOMUM DENTATUM. (Abstract) Jour. Parasitol. 22: 529.
(13) STOLL, NORMAN R.
1923. INVESTIGATIONS ON THE CONTROL OF HOOKWORM DISEASE. XV. AN EFFECTIVE METHOD OF COUNTING HOOKWORM EGGS IN FECES. Amer. Jour. Hyg. 3: 59–70.
(14) TAYLOR, E. L.
1935. PARASITIC GASTRITIS: THE CAUSES UNDERLYING ITS DEVELOPMENT IN SHEEP. Vet. Rec. 15: 1339–1344. [Reprinted from Jour. Min. Agr. [Gt. Brit.] 42: 647–657.]

EFFECT OF INFESTATION WITH THE NEMATODE COOPERIA CURTICEI ON THE NUTRITION OF LAMBS[1]

By JOHN S. ANDREWS[2]

Assistant zoologist, Zoological Division, Bureau of Animal Industry, United States Department of Agriculture

INTRODUCTION

Three major hypotheses have been advanced to explain the manner in which metazoan parasites living in the alimentary tract of vertebrates harm their hosts. According to one hypothesis, parasitic worms produce toxic substances which are absorbed by the host and cause the resulting pathological condition. Schwartz (*14*)[3] reviewed the literature on this subject and published the results of experiments which showed that extracts prepared from the tissues of five species of parasitic nematodes and one species of cestode contained hemolytic substances. Schwartz concluded that hemolytic and other hemotoxic secretions of parasitic worms were probably of etiological importance in parasitic disease.

According to a second hypothesis, the abstraction of blood by parasitic worms induces an anemic condition similar to that produced by chronic hemorrhage. Flu (*4*) did not find hemolysins in the tissues of the dog hookworm (*Ancylostoma caninum*). Like previous investigators, he found, however, an anticoagulin in the anterior portion of the worms. Assuming that the anticoagulin allowed profuse hemorrhage to occur, Flu inclined to the theory that chronic hemorrhage was the cause of anemia in hookworm disease in dogs. Wells (*20*, *21*) by direct observation demonstrated that *A. caninum* was a persistent bloodsucker. Following up the observation of Wells, Foster and Landsberg (*5*) made a study of the changes in the blood of dogs experimentally infested with *A. caninum*. They concluded that the symptoms of hookworm disease in dogs resulted from a blood loss at the site of the injuries produced by the worms in the intestinal mucosa. These workers were able to reproduce similar symptoms in uninfested dogs by periodic bleeding. Fourie (*6*) concluded from observations on the blood of sheep infested with the stomach worm (*Haemonchus contortus*), that the ingestion of blood by these worms and the resultant bleeding from the wounds produced by the parasites were the causes of the anemia associated with *Haemonchus* infection, this anemia being similar to that produced by severe hemorrhage.

According to a third hypothesis, parasitic worms produce antienzymes which inhibit the action of the enzymes in the digestive juices of the host, thus producing a state of chronic malnutrition. Stewart (*17*) and Shearer and Stewart (*15*) investigated the metabo-

[1] Received for publication March 11, 1938; issued September 1938.
[2] The writer expresses his appreciation to R. E. Davis, W. R. Kauffman, Thomas Jackson, J. M. Spadola, and C. O. Miller, of the Animal Nutrition Division, Bureau of Animal Industry, who did the chemical work connected with this investigation. He also acknowledges his indebtedness to Paul E. Howe and N. R. Ellis, of the same Division, for their cooperation in carrying out this study.
[3] Italic numbers in parentheses refer to Literature Cited, p. 360.

lism of heavily parasitized lambs. Stewart assumed that these lambs contained infestations similar to those in lambs from the same general area that had been examined post mortem and found to harbor the following nematodes: *Haemonchus contortus, Ostertagia circumcincta, Trichostrongylus extenuatus, Bunostomum trigonocephalum, Cooperia curticei, Nematodirus filicollis, Strongyloides papillosus, T. colubriformis, T. vitrinus,* and *Trichuris ovis.* The average number of worm eggs passed by a lamb in a 24-hour period was used by these authors as an index of the relative number of nematodes present in the host animal during each nutrition period of 14 days. Stewart reported that as the daily average number of worm eggs decreased, the digestibility coefficients of the crude-protein and crude-fiber portions of the ration increased. Shearer and Stewart found that more calcium and phosphorus were stored by lambs passing relatively small numbers of worm eggs than by those passing relatively large numbers of eggs. Stewart suggested that the inability of the infested lambs to make use of these components of the ration might be due to an antienzyme, which he demonstrated in the tissues of the parasites (*18*). According to Stewart, this antienzyme or "nezyme" might conceivably be present during heavy infestations in a quantity sufficient to impair seriously the function of the enzymes in the digestive juices of the host, and thus produce the condition of chronic malnutrition so characteristic of parasitic infestations.

Since the last-mentioned observations were made on lambs harboring a mixed infestation of nematodes, it occurred to the writer that a study of the metabolism of lambs infested with single species of parasites might yield useful information. Accordingly, the present investigation was undertaken at the Agricultural Research Center at Beltsville, Md.

MATERIALS AND METHODS

Four pairs of cross-bred Hampshire-Southdown wether lambs were selected for this work. Males were used because the separate collection of urine and feces in animals of this sex was a relatively simple matter. Lambs 60 and 61 (pair No. 1) and lambs 62 and 63 (pair No. 2) were born March 2, 1934, weaned the first week in May, and placed on experiment June 18, 1934. Lambs 64 and 65 (pair No. 3) were born March 26, 1934, weaned the last week in May, and placed on experiment July 16, 1934. These three pairs of lambs were born at the Agricultural Research Center, Md. Lambs 101 and 102 (pair No. 4) were received from the University of Maryland, College Park, Md., May 12, 1936, when about 10 days old, and were bottle-fed cow's milk until they were approximately 2 months old. They were then taken off cow's milk, given the feed mixture used during the experiment, and placed on experiment about a month later, namely, August 3, 1936.

Prior to the experiment the lambs were kept in individual wire pens having concrete floors raised approximately 2 feet above the ground. Adjacent to and connected with the pens at the same floor level were cinder-block houses in which metabolism cages were built. These pens were located in a wooded lot which afforded some protection from the sun. All the lambs were kept in the metabolism cages during the entire experiment, except when they were removed for

short periods for various reasons, such as weighing and collection of feces.

The metabolism cages used in the experiments were 5½ feet long and 2 feet wide. The walls of the cinder-block houses formed two sides of the cages; on the other two sides wire fencing was stretched over wooden frames placed so as to confine the lambs to the heavy wire platform shown in figure 1, *a*. This platform was made of ½-inch mesh wire cloth and was supported by an iron frame (*b*). A funnel-shaped trough (*c*) made of galvanized iron was suspended under this platform and served to guide the urine into a metal reservoir (*d*), containing a little toluene. A conical screen (*e*) which could be easily removed for cleaning was placed inside the trough over the small opening of the funnel to prevent the latter from becoming clogged. The feces were allowed to accumulate in a muslin bag made especially for the purpose. The bag was held over the anus of the lamb by means of muslin bands fastened to a shoulder piece made from a strip of burlap bag. The feed and water containers (*f*) and (*g*) were placed side by side at one end of the platform near the door of the cinder-block house where they were readily accessible and were so built and located that the lamb could not scatter the feed easily or spill the water into the funnel and thus dilute the urine.

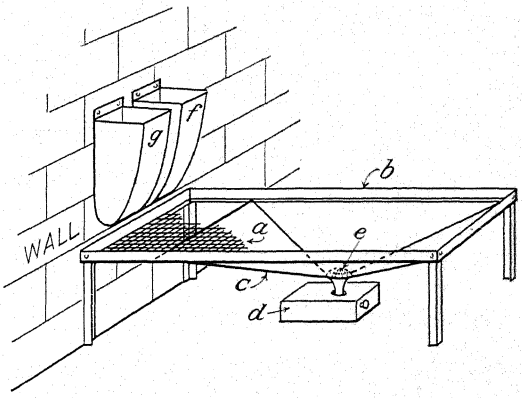


FIGURE 1.—Wire platform (*a*) and supplementary equipment of metabolism cage used in experiments; *b*, iron frame; *c*, funnel-shaped trough; *d*, metal reservoir; *e*, conical screen; *f* and *g*, feed and water containers.

For the purpose of infecting the experimental lambs, specimens of *Cooperia curticei* were collected from the small intestines of sheep slaughtered at an abattoir in Washington, D. C. The females were carefully washed in physiological saline solution until they were entirely free of debris, cut up with a pair of fine scissors, and the liberated eggs were cultured in a mixture of sterile sheep feces and granular bone charcoal moistened with distilled water. This mixture was put into Petri dishes 20 cm in diameter. The dishes were covered with the lids and left for about 7 days at room temperature. The infective larvae which developed from the eggs were then removed from the culture by means of the Baermann apparatus, and were administered in water by means of a funnel and rubber tube to a lamb that had been raised in a clean cage and was free from nematode parasites, except as noted later. When this lamb began to pass eggs of *C. curticei* 18 days after the first administration of larvae, the feces were collected, mixed with bone charcoal, and cultured in large Petri dishes for 7 days. The larvae were then removed from the cultures, counted, and administered daily to one member of each pair of the experimental lambs (Nos. 61, 63, 65, and 102). When these lambs

began to pass eggs of *C. curticei*, the feces were collected, treated as indicated previously, and the larvae used to supplement the infestation already present.

At the beginning of the experiment all the lambs were found to be infested with the nematode, *Strongyloides papillosus*. They had probably become infested from the feces of the ewes before they were weaned. Although the larvae of this nematode were not entirely eliminated from the doses administered to the infested lambs, they were greatly reduced in numbers by allowing the larvae obtained from the cultures to stand in water for a day or so at room temperature. Most of the larvae of *S. papillosus* became very sluggish or died, whereas the ensheathed larvae of *Cooperia curticei* were apparently unaffected by the exposure. No other extraneous nematode eggs or larvae were found in the feces of the sheep involved in this experiment.

The progress of the infestations in the host animals was followed by counting the number of worm eggs passed in the feces of the infested lambs from time to time by using the Lane direct centrifugal flotation and the Stoll dilution methods. The number of eggs per gram of feces so obtained was then multiplied by the number of grams of feces passed during the 24-hour period represented by the sample. The experiment was divided into nutrition periods 14 days in length (with the exception of one period of 13 and one of 18 days). The results of these observations were expressed in terms of the average number of worm eggs passed per 24 hours during each nutrition period.

When approximately 2 months old, each lamb was given about 2 pounds daily of the following mixture: Alfalfa meal, 27 pounds; ground oats, 10 pounds; corn meal, 5 pounds; and bran, 3 pounds. One-half pound of salt was added to the mixture for lambs 101 and 102. In addition, all lambs were given an ample supply of fresh water.

The mixture fed to the first three pairs of lambs had a nutritive ratio [4] of 1:3.9, and that fed to the last pair had a nutritive ratio of 1:4.4. The constituents of the mixture were ground so that selective feeding on the part of the lambs could not occur. At the time the feed was mixed, samples were removed for chemical analysis, put into airtight jars, and identified by a number. The remainder of the mixed feed was put into No. 6 paper bags, each bag containing sufficient feed (weighed to the nearest gram) for a single feeding of one lamb. These bags were stored in a barn loft in large metal containers, and at feeding time one bag for each lamb was removed and its contents dumped into the appropriate feed hopper. From time to time the quantity of feed contained in each bag was increased as the weight of the lambs increased.

At the end of each nutrition period the lambs were weighed before they were fed and after the bag containing the feces had been emptied. While the lambs were being weighed, the feed scattered about each cage was collected and later air-dried for 7 days. Once each week the drinking water container was emptied and the feed in it removed and air-dried for 7 days. These residues, which were not analyzed, were weighed to the nearest gram, and their weight subtracted from the total quantity of feed given to each lamb during a particular

[4] The ratio of the quantity of digestible protein to the quantity of digestible fat (×2.25) and carbohydrate in a given food substance.

nutrition period. The remainder was considered the actual quantity of feed ingested by the lamb during that period.

The feces and urine were collected at approximately the same hour each morning. The urine funnel was washed in running water after the removal of the sample. One-fifth of the weight of the daily feces from each lamb and one-tenth of the daily volume of urine from each lamb were put into separate ½-gallon fruit jars. These jars were sealed and stored in a refrigerator. As a preservative, a little toluene was added to the contents of the jars containing the urine. Later in the experiment it was found desirable to filter the urine through Whatman No. 1 filter paper in order to remove particles of debris.

The fecal samples from each lamb were thoroughly mixed in a mortar and 1 pint of the composite sample was put into an airtight jar. The urine samples from each lamb were thoroughly stirred and 1 liter of the composite sample was put into a stoppered bottle.

Quantitive analyses were made of the feed, feces, and urine samples and of the right femur of each lamb. When the analyses were completed, the apparent digestibility coefficients of each constituent of the feed were computed, and the amounts of nitrogen, calcium, and phosphorus stored by each lamb were ascertained. In connection with the work on the energy metabolism, the heat value of the feed mixture was calculated from the heat value of digestible protein, carbohydrate, and fat, as given by Morrison (*11*), and from the total digestible nutrients, obtained experimentally. The surface area of each lamb was calculated from its average weight during each nutrition period by the use of the formula ($S=0.139W^{0.501}$), of Ritzman and Colovos (*13*), for lambs less than 1 year of age. The energy necessary for the maintenance of each lamb was computed from the average value, 1,598 calories per square meter per 24-hour period, calculated from data given by Ritzman and Benedict (*12*), for lambs less than 1 year of age.

The four pairs of lambs were slaughtered at the end of 126, 126, 125, and 88 days, respectively. The thoracic and abdominal viscera were removed, weighed, and examined macroscopically for parasites and lesions.

Additional observations were made on body temperature, on the consistency of the feces, and on the cellular elements and hemoglobin of the blood. The results of the blood study have already been reported (*1*).

DATA OBTAINED

INFESTATIONS OF LAMBS WITH COOPERIA CURTICEI

The numbers of infective larvae of *Cooperia curticei* administered to one lamb of each pair are shown in table 1.

TABLE 1.—*Numbers of infective larvae of Cooperia curticei administered to one lamb of each pair*

Lamb No.	Daily feedings	Larvae administered per day	Total larvae administered	Lamb No.	Daily feedings	Larvae administered per day	Total larvae administered
	Number	*Number*	*Number*		*Number*	*Number*	*Number*
61	126	500	63,000		55	250	
63	84	1,000	105,000	65	36	500	65,750
	42	500			34	1,000	
				102	27	5,000	135,000

That the larvae of *Cooperia curticei* were able to establish themselves in the lambs is shown by the average number of worm eggs passed per 24 hours during each nutrition period (table 2). These data show that the parasites were present in considerable numbers in the infested lambs during the greater part of the experiment. The number of worm eggs contained in the feces of the control lambs was so small as to be negligible.

The number of each species of parasite found post mortem in the experimental lambs is shown in table 3.

TABLE 2.—*Number of worm eggs passed per 24 hours by the infested lambs during each nutrition period*

Lamb No.	Worm eggs passed per 24 hours in nutrition period No.—								
	1	2	3	4	5	6	7	8	9
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
61	0	71, 674	410, 870	1, 547, 500	1, 616, 233	2, 317, 667	1, 288, 900	2, 441, 600	1, 609, 960
63	0	412, 328	1, 510, 400	1, 440, 950	1, 277, 400	1, 403, 300	1, 100, 533	1, 370, 500	1, 999, 960
65	0	293, 400	436, 466	229, 800	262, 950	0	38, 625	25, 630	237, 730
102	0	0	846, 666	2, 018, 533	4, 215, 733	2, 696, 625			

As indicated in table 3, a number of species of nematodes in addition to *Cooperia curticei* were present in the experimental lambs. These extraneous species were probably acquired before the beginning of the experiment. *Strongyloides papillosus* was present in both the control and infested lambs. This fact, together with the relatively small numbers recovered, seemed to justify the elimination of this species as having influenced the results of the experiment. A few specimens of *Haemonchus contortus*, *Nematodirus spathiger*, and *Trichuris ovis* were also recovered, but since these nematodes were so few in number they too were considered of little importance in having any influence on the result of the experiment. It should be emphasized that although both the infested and control lambs harbored other species of parasites, the latter did not harbor specimens of *C. curticei*.

TABLE 3.—*Parasites recovered post mortem from the infested and control lambs*

INFESTED LAMBS

Lamb No.	*Cooperia curticei*	*Strongyloides papillosus*	*Nematodirus spathiger*	*Haemonchus contortus*	*Trichuris ovis*
	Number	*Number*	*Number*	*Number*	*Number*
61	8, 018	222	5	0	2
63	6, 919	418	4	1	0
65	455	184	1	0	0
102	25, 033	0	0	24	3
Average	10, 106. 3	206. 0	2. 5	6. 3	1. 3
Standard deviation	±10. 495. 6	±171. 4	±2. 4	±12. 3	±1. 5

CONTROL LAMBS

60	0	18	3	0	0
62	0	321	5	0	0
64	0	294	3	0	0
101	0	0	0	0	0
Average	0	158. 3	2. 8	0	0
Standard deviation	0	±172. 9	±2. 1	0	0

FEED CONSUMPTION AND WEIGHTS OF THE LAMBS

The weights of the lambs at the beginning and at the end of the experiment and the quantity of feed consumed are given in table 4. These data show that although the infested and control lambs of each pair consumed approximately the same quantity of feed, the control lambs gained approximately 4 pounds more than the infested lambs. Furthermore, the infested lambs required an average of 76.7 pounds more feed than the control lambs to produce a gain of 100 pounds. This difference was found to be statistically significant as it was 13.9 times its standard deviation. The limit of statistical significance here as well as in all other instances dealt with in this paper is 3.182 times the standard deviation.

In an attempt to account for the difference in the ability of the two groups of lambs to gain weight, the following data were obtained:

TABLE 4.—*Body weights and feed consumption of the infested and control lambs*

Lamb No.	Approximate length of feeding period	Body weight		Gain in weight	Feed consumed	Feed consumed per 100 pounds of gain
		Initial	Final			
INFESTED LAMBS						
	Weeks	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
61	18	38.0	75.0	37.0	282.6	763.7
63	18	36.5	75.0	38.5	287.0	745.4
65	18	47.5	85.0	37.5	310.5	828.1
102	12.5	31.0	59.0	28.0	233.7	834.8
Average	16.6	38.3	73.5	35.3	278.5	793.0
Standard deviation	±2.8	±6.9	±10.8	±4.9	±32.3	±45.1
CONTROL LAMBS						
60	18	36.5	78.5	42.0	284.4	677.2
62	18	31.5	75.0	43.5	289.1	664.6
64	18	34.5	76.0	41.5	311.1	749.6
101	12.5	28.5	58.0	29.5	228.2	773.6
Average	16.6	32.8	71.9	39.1	278.2	716.3
Standard deviation	±2.8	±3.5	±9.4	±6.5	±35.2	±53.5

APPARENT DIGESTIBILITY COEFFICIENTS

The average apparent digestibility coefficients of the different constituents of the feed for both the infested and control lambs are given in table 5. A comparison of the averages given in this table shows that there were no significant differences in the apparent digestibility coefficients of any constituent of the feed in the two groups.

TABLE 5.—*Average apparent digestibility coefficients of the different constituents of the feed of the infested and control lambs*

INFESTED LAMBS

Lamb No.	Dry matter	Crude protein	True protein	Non-protein nitrogenous matter	Ether extract	Crude fiber	Nitrogen-free	Organic matter	Ash
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
61	66.68	75.47	74.89	78.64	65.48	39.73	78.22	68.38	46.65
63	67.05	75.07	74.50	78.02	66.14	39.49	79.10	68.77	46.84
65	65.64	70.90	71.08	69.97	67.48	39.44	77.91	77.91	44.49
102	80.11	82.73	83.36	78.66	73.51	57.54	86.35	81.31	69.86
Average	69.87	76.04	75.96	76.32	68.15	44.05	80.40	74.09	51.96
Standard deviation	±6.85	±4.91	±5.22	±4.24	±3.67	±8.99	±4.00	±6.52	±11.98

CONTROL LAMBS

60	68.73	76.19	75.08	82.37	68.46	41.95	80.46	70.36	49.66
62	66.96	75.53	75.61	74.94	66.06	38.08	79.11	68.56	48.21
64	64.81	70.66	70.35	72.83	67.91	39.22	76.75	66.69	42.68
101	77.79	81.48	82.23	76.16	71.87	57.38	84.77	79.17	65.76
Average	69.57	75.97	75.82	76.58	68.58	44.16	80.27	71.20	51.58
Standard deviation	±5.71	±4.43	±4.89	±4.10	±2.42	±8.96	±3.42	±5.52	±9.91

NITROGEN AND MINERAL METABOLISM

The data on the nitrogen and mineral metabolism of the infested and control lambs are given in tables 6 and 7, respectively. As was the case with the apparent digestibility coefficients of the feed, there are no significant differences in the nitrogen metabolism of the two groups.

TABLE 6.—*Nitrogen metabolism of the infested and control lambs*

Infested lambs					Control lambs				
Lamb No.	Nitrogen stored	Urinary nitrogen excreted	Creatinine in urine	Hippuric acid in urine	Lamb No.	Nitrogen stored	Urinary nitrogen excreted	Creatinine in urine	Hippuric acid in urine
	Percent[1]	*Percent*[1]	*Grams*[2]	*Grams*[2]		*Percent*[1]	*Percent*[1]	*Grams*[2]	*Grams*[2]
61	25.97	49.58	2.70	15.75	60	28.78	47.34	2.08	14.11
63	26.38	48.57	.97	14.71	62	25.84	49.30	1.75	16.14
65	25.27	45.10	1.71	16.83	64	27.53	43.11	1.14	16.62
102	50.22	32.76	9.63	-------	101	46.90	34.89	4.12	-------
Average	31.96	44.00	3.76	15.76	Average	32.26	43.66	2.27	15.62
Standard deviation	±12.18	±7.74	±3.98	±1.06	Standard deviation	±9.83	±6.39	±1.29	±1.33

[1] Percent of total intake.
[2] Average per nutrition period.

Although the percentages of calcium and phosphorus stored by the infested lambs of the first three pairs were less than those stored by the corresponding control lambs, decidedly different results were obtained with the fourth pair of lambs. Lamb 102 was more heavily infested than lambs 61, 63, and 65 on post mortem (table 3), but it

appears that the infestation did not affect the mineral metabolism of the host. The writer is unable to account for the difference, in the storage of these elements, obtained between the infested lamb of the fourth pair and those of the first three pairs. Evidence that no serious modification of the mineral metabolism occurred in any of the infested lambs was provided by the bone analyses, which showed no significant differences in mineral content.

TABLE 7.—*Calcium and phosphorus stored in the body and the ratio of fat-free organic matter to ash in the right femur in infested and control lambs*

Lamb No.	Infested lambs			Lamb No.	Control lambs		
	Calcium	Phosphorus	Ratio of fat-free organic matter to ash in right femur		Calcium	Phosphorus	Ratio of fat-free organic matter to ash in right femur
	Percent [1]	*Percent* [1]	*1:*		*Percent* [1]	*Percent* [1]	*1:*
61	18.28	−2.90	1.625	60	24.20	+0.63	1.714
63	17.07	−7.31	1.510	62	22.73	−1.66	1.647
65	16.03	−7.19	1.520	64	17.38	−3.60	1.550
102	43.00	+40.24	1.406	101	40.63	−26.74	1.363
Average	23.60	+5.71	1.515	Average	26.24	+5.21	1.569
Standard deviation	±12.97	±23.11	±.09	Standard deviation	±10.20	±14.25	±.153

[1] Percent of total intake.

ENERGY METABOLISM

The energy value of the feed given to the first three pairs of lambs was 1.1 therms per pound (1 therm=1,000 calories), and of that given to the last pair was 1.178 therms per pound. By assuming that the heat production per square meter of body surface per 24-hour period was equal to the value found by Ritzman and Benedict (*12*) for lambs less than 1 year of age, and that it was the same for both the infested and control lambs, the heat production of all the lambs was calculated in order to determine whether or not the observed differences in the ability of the two groups to gain weight might be due to the increased energy requirements of the larger animals. This determination was particularly important in evaluating the difference in the ability to gain between lambs Nos. 64 and 65, whose initial weights were quite different. A summary of these calculations is given in table 8.

Table 8 shows that after the differences in the energy required for maintenance due to differences in the size of the lambs had been accounted for more energy per pound of live-weight increase was required by the infested lambs than by the control lambs. This difference was still significant, being 4.833 times its standard deviation. Whether this difference was due to the metabolism of the worms themselves or to some modification of the host's metabolism could not be determined experimentally. This question is discussed later.

TABLE 8.—*Energy metabolism of the infested and control lambs*

Lamb No.	Infested lambs			Lamb No.	Control lambs		
	Energy intake	Energy required for maintenance (heat production)	Energy required for growth per pound of live weight gained		Energy intake	Energy required for maintenance (heat production)	Energy required for growth per pound of live weight gained
	Therms	*Therms*	*Therms*		*Therms*	*Therms*	*Therms*
61	310.9	142.9	4.54	60	313.0	145.7	3.98
63	315.8	143.1	4.49	62	318.1	140.6	4.08
65	341.7	153.2	5.03	64	343.3	141.5	4.84
102	275.4	86.5	6.75	101	268.9	86.8	6.17
Average	311.0	131.4	5.20	Average	310.8	128.7	4.77
Standard deviation	±27.3	±30.3	±1.06	Standard deviation	±30.6	±28.0	±1.01

GENERAL CLINICAL OBSERVATIONS

The infested lambs showed no symptoms of parasitic infestations. There were no significant changes in body temperature. The infested lambs did not develop diarrhea, nor did they suffer from hemorrhage into the intestinal tract, as was shown by the absence of blood from the feces, as determined by the benzidine test.

DISCUSSION

The most significant result obtained in the present work was the apparent decrease in the ability of the infested lambs to convert their feed into gains in weight, although the lambs showed no clinical symptoms of parasitic infestation. This phenomenon could have been produced by a number of factors, operating either singly or in combination. Dehydration of the tissues could have played a part in producing this result. Although no moisture determinations were made on the carcasses, there appeared to be no good reason for assuming that the tissues of the lambs of one group contained less moisture than those of the lambs in the other group. Since the infested lambs did not develop high temperatures or suffer from hemorrhage or diarrhea during the experiment, and were given an abundant water supply, the assumption of a dehydration process is not warranted.

As shown by the data there were no differences in any of the apparent digestibility coefficients of the various constituents of the ration fed to the two groups of lambs, nor was there any significant difference in the amount of calcium and phosphorus stored by the lambs involved in this investigation. The results obtained by the writer fail to confirm those of Stewart (*17*), who found that there was a depression of the apparent digestibility coefficients of the crude-protein and crude-fiber components of the ration in nematode-infested lambs. The results of the writer's work are not in agreement with that of Shearer and Stewart (*15*), who reported that there was a decrease in the ability of the nematode-infested lambs to store calcium and phosphorus. Insofar as infestations with *Cooperia curticei* are concerned, the failure to discover any depression of the digestibility coefficients

of the infested lambs does not support Stewart's theory that parasites elaborate an antienzyme in quantities sufficient to interfere seriously with the digestive processes of the host.

The investigation reported in this paper failed also to show any increase in the nitrogen metabolism of the infested lambs. However, the results presented do show that infestations with the nematode (*C. curticei*) increased the energy metabolism of such lambs. The possibility that the increased energy metabolism might be accounted for by the metabolism of the worms themselves was given consideration. Weinland (*19*) first demonstrated that *Ascaris lumbricoides* used glycogen as a source of energy for its metabolic processes. Bondouy (*2*) found that *Sclerostomum equinum* (=*Strongylus equinus*) utilized fat as its source of energy, and Martini (*8*) found that the droplets in the intestine of *Oxyuris curvula* (=*O. equi*) were composed of fat and not glycogen. Even though *C. curticei* uses fat or glycogen or both in its metabolic processes, the worms are so small that it is doubtful whether such utilization of these substances could account for the increased metabolism observed in the infested lambs.

Bondouy (*2*) stated that the nervous symptoms seen in severe cases of parasitism were caused by the irritation of the intestinal mucosa by the worms. It is well known that if the nervous system is excited, more carbohydrate is burned by the animal and more energy is liberated. The presence of a worm infestation might conceivably produce a mild state of nervous tension that would be unnoticed. Such tension might be sufficient, however, to cause an increased metabolism. This condition would result in a decrease in the ability of the animal to gain in weight and would become apparent only after a period of weeks of careful observation under controlled conditions.

Recent studies on inflammation by Menkin and Warner (*9*) have shown that there is an increased carbohydrate metabolism in inflamed tissue. Since both the growth of the larvae in the intestinal mucosa with its attendant mechanical injury, and the tissue reaction of a resistant host to the foreign protein of the parasite, produce areas of inflammation, this condition may also contribute to the increased metabolism observed.

The increased metabolism of the infested lambs may be accounted for in still another way. Staub (*16*), Minot (*10*), and others have shown that the accumulation of guanidine in the blood of experimental animals was closely associated with wasteful carbohydrate metabolism. Harwood, Spindler, Cross, and Cutler (*7*) demonstrated that the guanidine content of the blood of rabbits increased after experimental infection with *Trichinella spiralis*. This association of guanidine accumulation with a nematode infestation, together with its demonstrated effect on carbohydrate metabolism, suggests the possibility that this substance may be one of the factors contributing to the result observed in this investigation.

Although Edgar (*3*), ascribed fatalities in goats to heavy infestations with *Cooperia curticei*, the writer has found this nematode relatively nonpathogenic for sheep. In connection with these conflicting observations, it should be emphasized that the lambs involved in the experiments reported in this paper were fed an adequate ration and were given excellent care. As a result, they were undoubtedly better able to resist the pathogenic effects of the nematode infection to which they were subjected than lambs not living under such ideal conditions.

The more or less accidental selection of *C. curticei* for this work has made possible the study of the effect of a nematode infestation, on the nutrition of the host, in the absence of such complicating factors as anemia and diarrhea, which so often accompany infections with the better known pathogenic nematodes, such as *Haemonchus contortus* and *Trichostrongylus colubriformis*. It has also made possible the demonstration that infestations with *C. curticei* have a measurable effect on the host, even when the latter is in excellent nutritional condition. This finding is of importance because it introduces evidence to show that a nematode infestation increases the cost of lamb production even though it may not be sufficiently heavy to produce clinical symptoms.

SUMMARY AND CONCLUSIONS

The nutrition of eight cross-bred Hampshire-Southdown wether lambs, four of which were experimentally infested with the nematode *Cooperia curticei* was studied in a paired feeding experiment.

The results obtained failed to confirm the finding of Stewart (*17*) that there was a depression of the digestibility of the crude-protein and crude-fiber components of the ration in the infested lambs. Nor was any evidence found to support the contention of Shearer and Stewart (*15*), that there was a decrease in the ability of the infested lambs to store calcium and phosphorus. The findings with reference to *Cooperia curticei* are not in accord with Stewart's theory that parasites elaborate an antienzyme in quantities sufficient to interfere with the digestive processes of the host.

The investigation shows that infestations with a relatively non-pathogenic nematode decrease the ability of the infested lambs to convert their feed into gain in weight, even when these lambs are in excellent nutritional condition and show no clinical symptoms of parasitic infestation. Evidence is presented to show that this decrease in efficiency is due to an increased energy metabolism, apparently resulting in reduced use of the feed for growth or gain.

The increased energy metabolism of the infested lambs may be accounted for by the nervous excitation of the host due to the irritation of the intestinal mucosa by the worms in the intestine, by the production of areas of inflammation wherever the mucosa was injured by the larvae and wherever a tissue reaction of the partially resistant host to the foreign protein of the parasite occurred, and by the possible, although undemonstrated, accumulation of guanidine in the blood of the infested lambs.

LITERATURE CITED

(1) Andrews, J. S.
1938. Observations on the cellular elements and hemoglobin in the blood of sheep infected with the nematode, Cooperia curticei. North Amer. Vet. 19 (2): 40–44.

(2) Bondouy, T.
1910. Chemie biologique du sclerostomum equinum. 58 pp. Paris. (These).

(3) Edgar, Grahame.
1936. Fatal effect of heavy infestation with cooperia curticei (railliet, 1893) in goats. Aust. Vet. Jour. 12 (2): 58–61.

(4) Flu, P. C.
1926. Investigations concerning the causes of anaemia in cases of ankylostomiasis. Acta Leidensia 1: 10–33. [English translation of paper by same author in Tijdschr. Vergelijk. Geneesk. 7 (1) 47–72. 1922.]

(5) FOSTER, A. O., and LANDSBERG, J. W.
1934. THE NATURE AND CAUSE OF HOOKWORM ANEMIA. Amer. Jour. Hyg. 20: 259–290, illus.

(6) FOURIE, P. J. J.
1931. THE HAEMATOLOGY AND PATHOLOGY OF HAEMONCHOSIS IN SHEEP. Union So. Africa Dept. Agr., Dir. Vet. Serv. and Anim. Indus. Ann. Rept. 17: 495–572, illus.

(7) HARWOOD, P. D., SPINDLER, L. A., CROSS, S. X., and CUTLER, JESSIE T.
1937. GUANIDINE RETENTION IN EXPERIMENTAL TRICHINOSIS OF RABBITS. Amer. Jour. Hyg. 25: 362–371.

(8) MARTINI, E.
1916. DIE ANATOMIE DER OXYURIS CURVULA. Ztschr. Wiss. Zool. 116: [137]–338, [339]–534, illus.

(9) MENKIN, VALY, and WARNER, CHARLOTTE R.
1937. STUDIES ON INFLAMMATION. XIII. CARBOHYDRATE METABOLISM, LOCAL ACIDOSIS, AND THE CYTOLOGICAL PICTURE OF INFLAMMATION. Amer. Jour. Path. 13: 25–43, illus.

(10) MINOT, A. S.
1931. THE MECHANISM OF THE HYPOGLOCEMIA PRODUCED BY GUANIDINE AND CARBON TETRACHLORIDE POISONING AND ITS RELIEF BY CALCIUM MEDICATION. Jour. Pharmacol. and Expt. Ther. 43: 295–313.

(11) MORRISON, F. B.
1936. FEEDS AND FEEDING, A HANDBOOK FOR THE STUDENT AND STOCKMAN. Ed. 20, unabridged, 1,050 pp., illus. Ithaca, N. Y.

(12) RITZMAN, E. G., and BENEDICT, F. G.
1930. THE ENERGY METABOLISM OF SHEEP. N. H. Agr. Expt. Sta. Tech. Bull. 43, 23 pp., illus.

(13) ——— and COLOVOS, N. F.
1930. SURFACE AREAS OF SHEEP. N. H. Agr. Expt. Sta. Cir. 32, 8 pp.

(14) SCHWARTZ, BENJAMIN.
1921. HEMOTOXINS FROM PARASITIC WORMS. Jour. Agr. Research 22: 379–432.

(15) SHEARER, GEORGE D., and STEWART, JAMES.
1933. THE EFFECTS OF NEMATODE INFESTATIONS ON THE METABOLISM OF THE HOST. PART III. THE EFFECTS OF NEMATODE INFESTATIONS ON MINERAL METABOLISM. Cambridge Univ., Inst. Anim. Path. Rept. Dir. (1932–33) 3: 87–97.

(16) STAUB, H.
1928. EXPERIMENTELLE UNTERSUCHUNGEN ÜBER SYNTHALINWIRKUNG. Ztschr. Klin. Med. 107: 607–658.

(17) STEWART, JAMES.
1933. THE EFFECTS OF NEMATODE INFESTATIONS ON THE METABOLISM OF THE HOST. PART I. METABOLISM EXPERIMENTS. Cambridge Univ., Inst. Anim. Path. Rept. Dir. 3: 58–76. Appendix. Digestion Tables, pp. 98–129.

(18) ———
1933. THE EFFECTS OF NEMATODE INFESTATIONS ON THE METABOLISM OF THE HOST. PART II. THE ISOLATION OF A SUBSTANCE CAPABLE OF INHIBITING ENZYME ACTION. Cambridge Univ., Inst. Anim. Path. Rept. Dir. 3: 77–86.

(19) WEINLAND, ERNST.
1901. ÜBER DEN GLYKOGENGEHALT EINIGER PARASITISCHER WÜRMER. Ztschr. Biol. 41: [69]–74.

(20) WELLS, HERBERT S.
1931. AN OBSERVATION WHICH SUGGESTS AN EXPLANATION OF THE ANEMIA IN HOOKWORM DISEASE. Science 73: 16–17.

(21) ———
1931. OBSERVATIONS ON THE BLOOD SUCKING ACTIVITIES OF THE HOOKWORM, ANCYLOSTOMA CANINUM. Jour. Parasitol. 17: [167]–182, illus.

A METHOD OF DETERMINING ROOT KNOT RESISTANCE IN BEANS AND COWPEAS IN THE SEEDLING STAGE [1]

By KEITH C. BARRONS

Assistant vegetable breeder, Alabama Agricultural Experiment Station

INTRODUCTION

In connection with a breeding program designed to develop improved root-knot-resistant beans (*Phaseolus vulgaris* L.), cowpeas (*Vigna sinensis* (*Torner*) Savi), and lima beans (*P. lunatus* var. *macrocarpus* Benth.) the need was felt for an economical and accurate method of determining root knot resistance. Field observations are sometimes undependable, because of the uneven distribution of the root knot nematode (*Heterodera marioni* (Cornu) Goodey) even in soils considered to be badly infested. Furthermore, such observations are costly in a breeding program in which extensive trials are made and large segregating populations are grown. In the experiments reported in this paper an attempt was made to develop an inexpensive and accurate method of determining root knot resistance in the seedling stage under controlled greenhouse conditions.

MATERIALS AND METHODS

Bean and cowpea varieties of known degrees of resistance were used in these experiments. They included Alabama No. 1 and Alabama No. 2 beans, which were found by Isbell [2] to be highly resistant to root knot; Hopi 155 lima bean, which is noticeably less susceptible than other lima varieties under field conditions; and Conch cowpea, reported by Isbell [3] to be the most resistant of the edible cowpea varieties. A number of common varieties of the three species known to be susceptible on the basis of field observations were also used.

The plantings were made in a raised greenhouse bench 4 inches deep. As nematode injury is commonly believed to be greater in sandy soils, a sandy loam was used throughout the experiments. All plantings were made in soil to which a mixed fertilizer in quantities sufficient for good growth had been applied. The soil was kept moist at all times since Godfrey [4] found that maximum root knot development occurred in moist but well-drained soils.

A minimum soil temperature of 28° C., which is sufficiently high for root knot development, was maintained during cool weather by the use of an electric soil-heating cable buried 1 inch from the bottom of the bench. The temperature was controlled by a soil-heating thermostat.

[1] Received for publication December 10, 1937; issued September 1938.

[2] ISBELL, C. L. NEMATODE-RESISTANCE STUDIES WITH POLE SNAP BEANS. Jour. Heredity 22: 191-198, illus. 1931.

[3] ISBELL, C. L. NEMATODE INJURY TO THE ROOTS OF TABLE VARIETIES OF COWPEAS. Ala. Agr. Expt. Sta. Ann. Rept. 45: 29-30. 1934.

[4] GODFREY, GEORGE H. EFFECT OF TEMPERATURE AND MOISTURE ON NEMATODE ROOT-KNOT. Jour. Agr. Research 33: 223-254, illus. 1926.

Root knot galls from bean, lima bean, and tomato plants grown in the field were used as a source of inoculum. The galls were cut into small pieces before being applied to the soil.

EXPERIMENTAL RESULTS

In these investigations an attempt was made to experimentally establish the following: (1) Satisfactory conditions of soil and temperature for differential root knot development, (2) the best method of inoculation, (3) the optimum time and the most efficient method of classification, (4) a satisfactory arrangement of plantings, and (5) the agreement of results between seedling tests and field observations on adult plants.

SOIL AND TEMPERATURE CONDITIONS

In preliminary tests inoculum was added to field soil known to be badly infested, field soil which was practically free from nematodes, and soil which had been steam-sterilized. No differences in the rate or degree of root knot development were apparent. These results indicate that when soil is thoroughly inoculated the nematodes already present do not create a more complete infestation. Sterilized soil is probably best, because pathogens other than the root knot nematode will be largely eliminated. Furthermore, nodulation that might be confused with root knot galls without careful observation will be reduced.

Godfrey,[5] working with crops other than beans and cowpeas, found that root knot developed abundantly at temperatures as high as those at which the host plant would thrive. In some cases he found that there was a reduction in the amount of the disease above 30° C., but this was usually traceable to a decrease in total root growth. In his experiments there was a noticeable decrease in root knot development when the soil temperature was lowered to 19° or below. The optimum temperature for maximum infection was found to vary somewhat with different species of plants but ranged between 22° and 30°.

The experiments reported in this paper were conducted largely during July, August, and September. Records taken at intervals during the experiments indicated that the soil temperature close to the bottom of the greenhouse bench ranged from 25° to 32° C., occasionally going higher for a few hours on exceptionally hot days. As excellent root knot development took place during this entire period, it was concluded that greenhouse temperatures during the summer months were satisfactory. Tests conducted during the fall did not prove as satisfactory because of the slower rate of root knot development. A soil-heating cable was found useful in maintaining a uniform soil temperature; however, in no case were results after October 1 as satisfactory as during the summer months. Greenhouse equipment for controlling both soil and air temperatures should be available if such tests are to be conducted during the cooler seasons of the year.

METHOD OF SOIL INOCULATION

Inoculum prepared from root knot galls of common bean, lima bean, and tomato plants was used with equal success. Steiner [6] has pointed

[5] GODFREY, GEORGE H. See footnote 4.

[6] STEINER, G. THE PROBLEM OF HOST SELECTION AND HOST SPECIALIZATION OF CERTAIN PLANT-INFECTING NEMAS AND ITS APPLICATION IN THE STUDY OF NEMIC PESTS. Phytopathology 15: 499-534, illus. 1925.

out cases of host specialization and host preference in various nematode species including *Heterodera marioni*. In view of such possible differences in various populations of *H. marioni*, preliminary tests of any new source of inoculum would undoubtedly be a wise procedure. In these experiments inoculum was prepared by cutting the diseased portions of the roots being used into small pieces. Care was taken at all times not to allow the roots or the prepared inoculum to dry out.

An experiment was conducted to determine whether the stage of development of the root knot tissue used as a source of inoculum would affect the results. Two lots of inoculum were prepared, one from young root knot tissue taken from tomato plants which were setting their first fruit and the other from large galls removed from tomato plants which had been in production for 4 to 5 weeks. Equal quantities by weight of these two lots were applied to equal areas of soil in the greenhouse bench. At the end of 20 days seedlings of susceptible beans and cowpeas grown in the soil to which the old root knot tissue had been added, showed a higher degree of infection. Galls were both larger and more numerous than on seedlings grown in soil to which young root knot tissue had been applied. The success of a seedling test for root knot resistance depends on the presence in the inoculum of a large number of active larvae which will attack each portion of the root as it develops. Microscopic observations of inoculum from the two sources revealed that the old root knot tissue contained a larger number of active larvae and the young tissue a larger proportion of adults and eggs. Figure 1 illustrates the type of badly diseased adult lima bean root which was also found to be a good source of inoculum.

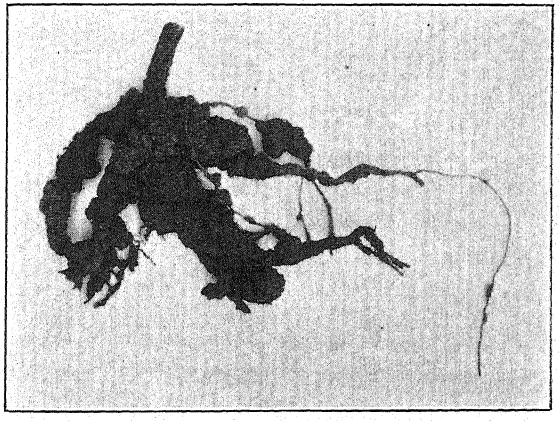


FIGURE 1.—Badly diseased root of Henderson Bush Lima. Such old root knot tissue contains innumerable active nematode larvae and is an excellent source of inoculum.

In some instance good results were obtained by using inoculum prepared from partially decayed root knot galls. On a few occasions, particularly late in the fall, such inoculum did not give good results even though on microscopic examination it was found to contain innumerable active nematode larvae.

Tyler [7] found that the ability of larvae from different sources to enter roots showed considerable variation. She concluded that such variation is related in part to the age of the larvae, which suggests that the inferiority of certain lots of inoculum prepared from decayed roots may be due to decreased vigor of the larvae rather than to a reduction in their number. As previously suggested, variation in

[7] TYLER, JOCELYN. REPRODUCTION WITHOUT MALES IN ASEPTIC ROOT CULTURES OF THE ROOT-KNOT NEMATODE. Hilgardia 7: [373]-388. 1933.

inoculum from any new source should be ascertained by preliminary tests in which varieties of known degrees of susceptibility are used as standards.

A comparison was made between the degree of root knot development when equal quantities of inoculum were applied by mixing with the soil and by placing in furrows beneath the seed. Satisfactory results were obtained by both methods; however, root knot galls were somewhat more evenly distributed when the furrow method was used. Fifty grams of inoculum per meter of row proved sufficient in all cases when prepared from large root knot galls.

PLANTING ARRANGEMENT

It was found satisfactory to space the rows 4 inches apart and the seeds 2 inches apart in the row. Wider spacing did not give a greater degree of root knot infection. When less space was allowed, the removal and separation of roots proved to be more difficult.

A test was conducted to determine whether the degree of root knot infection on any one variety would be affected by adjacent varieties. According to Steiner,[8] there is evidence to show that nematodes possess the ability to select certain host plants. This suggested that if single rows of different varieties were grown the larvae might be attracted to certain varieties, thus reducing the amount of infection in others. In this experiment three consecutive rows each of a number of varieties were planted. Comparisons were made between the degree of infection on the center and outside rows of each variety. No differences were noted. Further plantings of Alabama No. 1 and Kentucky Wonder beans and Conch cowpea in isolated sections of a greenhouse bench showed the same differences with respect to root knot injury as when planted in adjacent rows. It was, therefore, concluded that when sufficient inoculum is used single rows of different varieties may be planted close to each other without affecting the reliability of the results obtained.

TIME AND METHOD OF CLASSIFICATION

Experiments were conducted to determine at what stage in the development of the plant the greatest differentation occurs between resistant and susceptible varieties, and at what stage classifications can be made most rapidly and efficiently. The roots of several varieties of beans, cowpeas, and lima beans planted in inoculated soil on July 15 were examined 10 days after sowing and every 10 days thereafter until they were 40 days old.

These observations indicated that the distinction between resistant and susceptible varieties was greater 20 days after planting than after a 10-, 30-, or 40-day period. From observations on other plantings it was concluded that during the summer when greenhouse temperatures range upward from 25° C. and the rate of plant growth approaches the maximum, good classifications may be made from 20 to 25 days after planting. Plants from seed sown in late September and early October developed less rapidly, and it was found that greater distinction between resistant and susceptible varieties could be made after a period of from 25 to 30 days.

[8] STEINER, G. See footnote 6.

Although the resistant could be distinguished from the susceptible varieties as early as 10 days after planting, the differences were not so pronounced as at the end of a 20-day period. All varieties studied appeared to be subject to attack by the root knot nematode. A few galls were found on the most resistant strains, but they seldom amounted to more than small swellings. Galls on susceptible and resistant varieties were similar in appearance soon after infection took place, but on the former they continued to develop at a rapid rate and were often half a centimeter or more in diameter by the end of 20 days.

At the end of 30 days during the summer and after a 40-day period in all cases the various degrees of resistance could not be distinguished as readily as at the end of 20 days or shortly thereafter. These older plants had a larger mass of roots which were difficult to remove from the bench. In some instances nodulation had developed which made

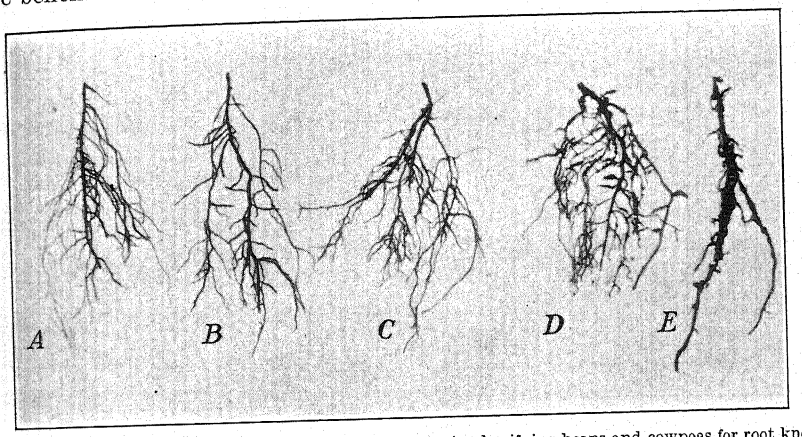


FIGURE 2.—Portions of roots of varieties used as standards in classifying beans and cowpeas for root knot resistance: *A*, (class 1) bean grown in nematode-free soil; *B*, (class 2) Alabama No. 1 bean; *C*, (class 3) Conch cowpea; *D*, (class 4) Surecrop Wax bean; *E*, (class 5) Kentucky Wonder bean.

a rapid classification difficult. At this stage the younger root growth was practically free from galls. This was probably due to most of the available nematode larvae having entered the roots during the early growth of the plant. Their life cycle then had to be completed; larvae had to enter the soil and migrate to new root growth before more galls could be formed.

Observational ratings based on the experimenter's judgment were used in classifying the material. Because of the coalescing of galls it proved impractical to use gall counts as an index of susceptibility. It was found that varieties could be conveniently grouped into five classes with respect to root-knot resistance and susceptibility as illustrated in figure 2. Plants grown in nematode-free soil were used as a standard for class 1. Alabama No. 1 and Alabama No. 2 beans which developed only a few slight swellings were the only varieties which were consistently placed in class 2. Conch cowpea is considered a good standard for class 3 in which it was usually placed. Root knot galls on this resistant variety were slightly larger and somewhat more numerous than on Alabama No. 1 and Alabama No. 2 beans. Surecrop Wax bean was found to be a good standard for

class 4. It possesses mild resistance both in the adult and the seedling stage. Practically all common varieties of beans, cowpeas, and lima beans are very susceptible and were placed in class 5. Among the cowpeas the Crowder varieties and Dixie Queen, and among the beans Kentucky Wonder and Bountiful were found to be highly susceptible. They may be used as standards for comparison in making root-knot-resistance determinations. Variations within class 5 probably exist. Galls on some susceptible varieties may be consistently larger and more numerous than on others; however, this was not considered significant from the standpoint of a method of resistance determination.

A statistical study was conducted to determine the extent of variation in root knot development among plants within one variety. This information was desired in order to determine whether accurate data concerning varietal resistance could be obtained by rating a population of 10 or more plants at once or whether a mean of individual-plant ratings would be more reliable. Four replications of eight varieties were used in this experiment. The roots of 10 plants chosen at random from each row were bundled together and designated by number. A rating was given each row based on the general appearance of its roots. On summarizing the data thus obtained it was found that in no case was there a difference in the rating given the four samples of the same variety. Roots of the 10 plants in each bundle were then rated individually. As four rows of each variety were observed in this way the means obtained were for 40 plants. Table 1 presents the ratings given the eight varieties by the two methods of observation.

TABLE 1.—*Root knot resistance of seedlings of representative bean, cowpea, and lima bean varieties as determined by two methods of classification*

Variety	Resistance classification by 10-plant method	Resistance classification by individual-plant method [1]	Variety	Resistance classification by 10-plant method	Resistance classification by individual-plant method [1]
Beans:			Cowpeas—Continued.		
Alabama No. 1	2	1.87±0.05	Large Cream Crowder	5	5.00±0.00
Alabama No. 2	2	1.92± .04	Dixie Queen	5	4.95± .03
Bountiful	5	4.97± .02	Lima beans:		
Cowpeas:			Hopi 155	5	4.55± .11
Conch	3	2.85± .06	Henderson Bush	5	5.00± .00

[1] Means are for 40 plants with variation expressed as standard error.

It will be noted that with the exception of Hopi 155 the means obtained by the individual-plant method were practically identical with the numerical ratings assigned when a 10-plant sample was taken as the unit of observation. Likewise with the exception of this one variety the means have a low standard error value, indicating that little variation was observed between plants. Hopi 155 has also been observed to be variable with respect to root knot resistance under field conditions. On the basis of these results it was concluded that when uniformly inoculated soil is used accurate readings may be made on pure lines by observing a population of 10 or more plants as a whole. Progeny of crosses and lines the purity of which has not been established will require individual plant observations.

AGREEMENT OF SEEDLING TESTS AND FIELD OBSERVATIONS

The results of field observations on the degree of root knot development on a number of varieties of beans, cowpeas, and lima beans were compared with those of greenhouse seedling tests. In the case of beans the agreement between seedling and adult plant observations was close. Alabama No. 1 and Alabama No. 2 were found to be highly resistant both in the adult and seedling stage. Surecrop Stringless Wax was only moderately susceptible while Kentucky Wonder and Bountiful were susceptible in both stages.

The agreement of seedling and adult readings was not as close in the case of cowpeas, yet the observations by the two methods were similar enough to make the seedling test of definite value in cowpea breeding work. Conch has been observed to be as free from root knot in the field as Alabama No. 1 and Alabama No. 2 beans; yet in the seedling tests it developed a few galls of intermediate size. Cream Crowder, Black Crowder, and Dixie Queen were susceptible in both field and seedling tests.

With lima beans no definite agreement was found between seedling and adult plant observations. As has been noted, Hopi 155 was somewhat variable in the seedling tests; however, it was definitely susceptible with only occasional plants appearing partially resistant. In the field this variety has been observed to be partially resistant with occasional plants appearing susceptible. Because of these differences in seedling and adult plant observations, the seedling test is probably of no immediate value in testing lima bean varieties. The fact that certain plants of Hopi 155 appeared mildly resistant in the seedling stage suggests that further selection within this variety might result in lines possessing some seedling resistance. Further research on the nature of root-knot resistance may explain why Hopi lima beans appear susceptible in the seedling stage and partially resistant in the field.

On the basis of these experiments it was concluded that the seedling method described is very accurate in determining root-knot resistance in the common bean. Although the agreement of adult-plant and seedling observations is not as close in the case of cowpeas as in beans very definite differences were observed between the most resistant and the susceptible varieties. There appears to be no reason why this method should not be used in a cowpea breeding program in which only the highly resistant individuals and lines are to be selected. The method is at present of no value in testing lima beans, as no significant differences in the degree of infection can be noted between varieties which show marked differences under field conditions.

SUMMARY

A method has been developed for determining root knot resistance in the seedling stage under controlled greenhouse conditions. The method is satisfactory for beans and cowpeas but unsatisfactory for lima beans.

A fertile sandy soil which had been previously sterilized and kept moist at all times was found to be a good medium for growing seedlings to be tested. Natural greenhouse temperatures prevalent during the summer months ranging upward from 25° C. were found to be satis-

factory for good root knot development, but during the fall artificial heat had to be applied to the soil.

Inoculum consisting of chopped rootk not tissue from badly diseased adult bean and tomato plants applied in furrows beneath the seed at the rate of approximately 50 g per meter of row gave the best results. Rows spaced 4 inches apart with seeds 2 inches apart in the row were found satisfactory.

It was found that the best classification could be made between 20 and 30 days after planting, depending on the rate of plant growth.

It was experimentally established that when pure lines are being tested observational ratings of a group of 10 or more plants as a whole are as accurate as ratings of individual plants. Varieties can be divided into five classes on the basis of their root knot resistance, plants grown in nematode-free soil, being taken as the standard for class 1, Alabama No. 1 and Alabama No. 2 beans for class 2, Conch cowpea for class 3, Surecrop Wax bean for class 4, and Kentucky Wonder or almost any common bean or cowpea variety for class 5.

HOOD AND SUPERNUMERARY SPIKE DEVELOPMENT IN BARLEY[1]

By O. T. BONNETT
Assistant chief in plant genetics, Illinois Agricultural Experiment Station

INTRODUCTION

The lemmas of Nepal barley (*Hordeum vulgare* L.) terminate in a trifurcated structure called a hood. The center lobe of the hood is an accessory flower having stamens and a pistil, but the lateral wings of the hood are only vegetative outgrowths of the margins of the lemma. When the hoods of mature spikes are examined, various degrees of development of the stamens and pistil can be found. Almost invariably the supernumerary florets of the hood are infertile, but Biffen (*2*)[2] states that he has found fertile florets in the hood and has grown plants from the grain that they produced.

Hooded barley was found, according to Harlan (*4*), in Nepal between the years 1830 and 1837. He believes that the hooded character probably originated as a mutation. Evidence supporting this assumption is based upon the discovery of a hooded mutant in the F_2 of a cross of Everest × Manchuria, two awned varieties. If this hypothesis is correct, the hooded character is an example of a dominant mutation.

Biffen (*2*), Hor (*5*), and Robertson (*8*) made crosses between hooded and awned varieties. In crosses of hooded with awned varieties of barley they found that hooded was dominant to awned, and that segregation occurred in the F_2 in the ratio of approximately 3 hooded to 1 awned.

The hooded character in barley is of interest for another reason. Arber (*1*) states that the origin of accessory spikelets from the lemma is contrary to one of the dicta of formal morphology, which is that the power of producing lateral shoots is confined to axes. However, in the case of hooded barley, the spikelet, a modified shoot, is produced from the lemma, a modified leaf.

A morphological study of the development of hooded barley is of interest from two standpoints. By studying the morphological development of the hood, it is possible, so far as external appearances will permit, to determine when the genes responsible for the development of the character begin to produce their effects, and to see what changes the genes produce. Furthermore, according to Arber (*1*), some morphologists have questioned whether the accessory flower actually does arise from the lemma as its axis. By following the developmental sequence a better idea can be obtained of the points of origin and parts initiated than can be obtained by studying the mature parts. This is because many times parts are initiated that never complete development and in the beginning of development the surrounding parts are not large enough to hide the point of origin of the parts being studied.

[1] Received for publication January 8, 1938; issued September 1938.
[2] Italic numbers in parentheses refer to Literature Cited, p. 377.

Supernumerary spikes were found on the stems of Nepal barley when the development of the hood was being studied. They had also been observed, previously, in greenhouse cultures of awned barley. The photomicrographs used to show this character were taken from awned barley.

MATERIALS AND METHODS

Nepal C. I. No. 595 was obtained from M. N. Pope, division of cereal crops and diseases, United States Department of Agriculture. This variety was used for a study of hood development.

The awned varieties in which supernumerary spikes developed were Spartan, Wisconsin Pedigree No. 5, and their F_2 progeny.

Supernumerary spikes were produced upon plants grown during the winter of 1935 and 1936 on a greenhouse bench filled with about 6 inches of soil. The plantings were made on November 22, 1935, and the plants from which the supernumerary spikes were dissected were sampled on February 21, 1936. The growing period extended through the short days of the winter, and during the period of spike differentiation, December 27 to February 21, the temperature was seldom above 65° F. and dropped to as low as 45° for short periods. The average minimum temperature was 53.2°, the average maximum temperature was 68.1°, and the average mean temperature was 60.8°.

The photomicrographic apparatus and the technique used in taking the photomicrographs were essentially the same as those described in an earlier paper (*3*). One exception was that special microlenses having a focal distance of 24 and 32 mm were used to take the photomicrographs instead of one side of a binocular microscope.

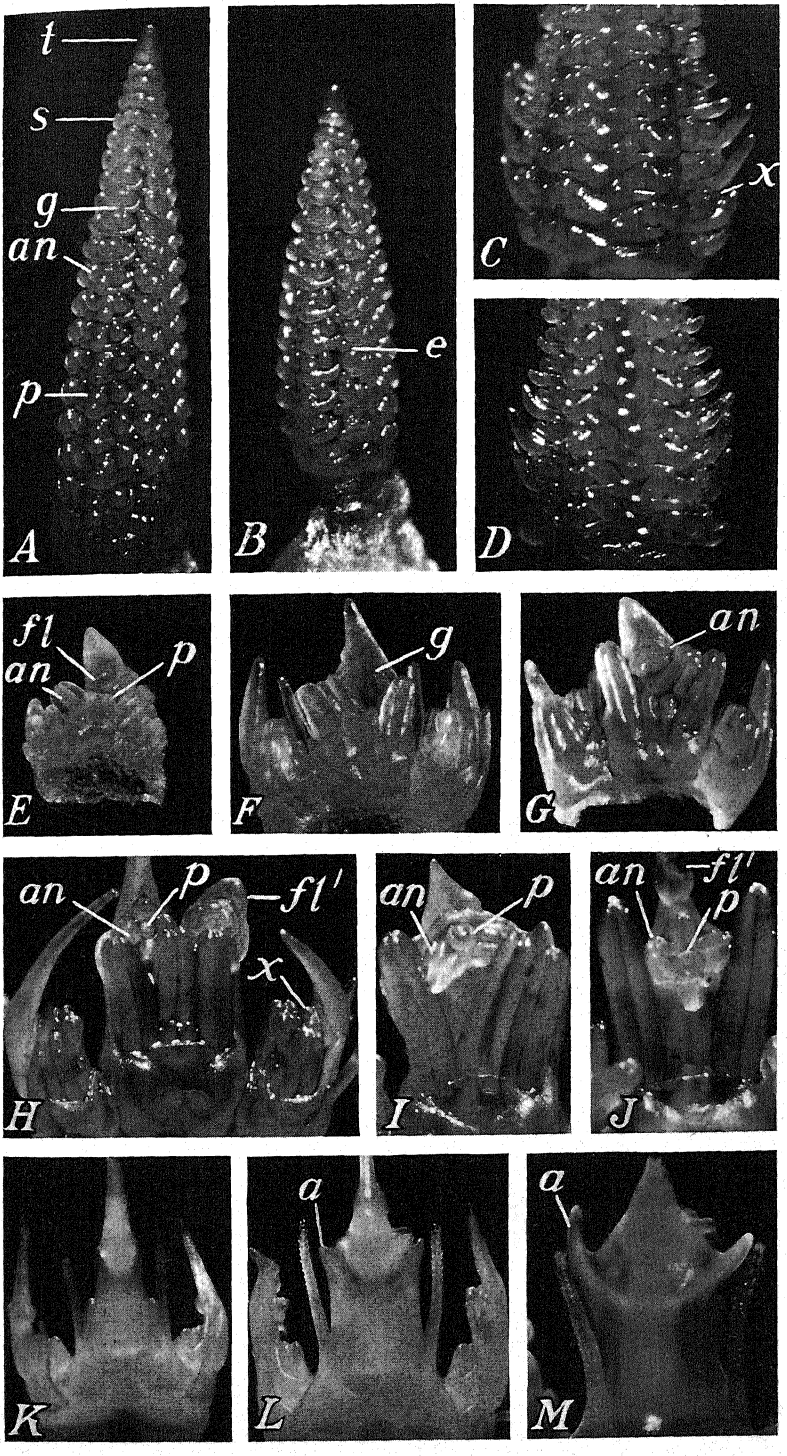
DEVELOPMENT OF THE HOOD

Since the hood develops from the lemma, the lemma must differentiate and attain some size before the hood primordium appears. An early stage in the development of a spike of hooded barley is shown in plate 1, *A*, and a spike of an awned barley is shown in plate 1, *B*. In both cases the lemmas of the central spikelets have differentiated and the tips of the lemmas have begun to elongate in the formation of the awn. At this stage the hood primordia have not differentiated and no differences in the appearance of the spikelets of the hooded and awned types can be observed.

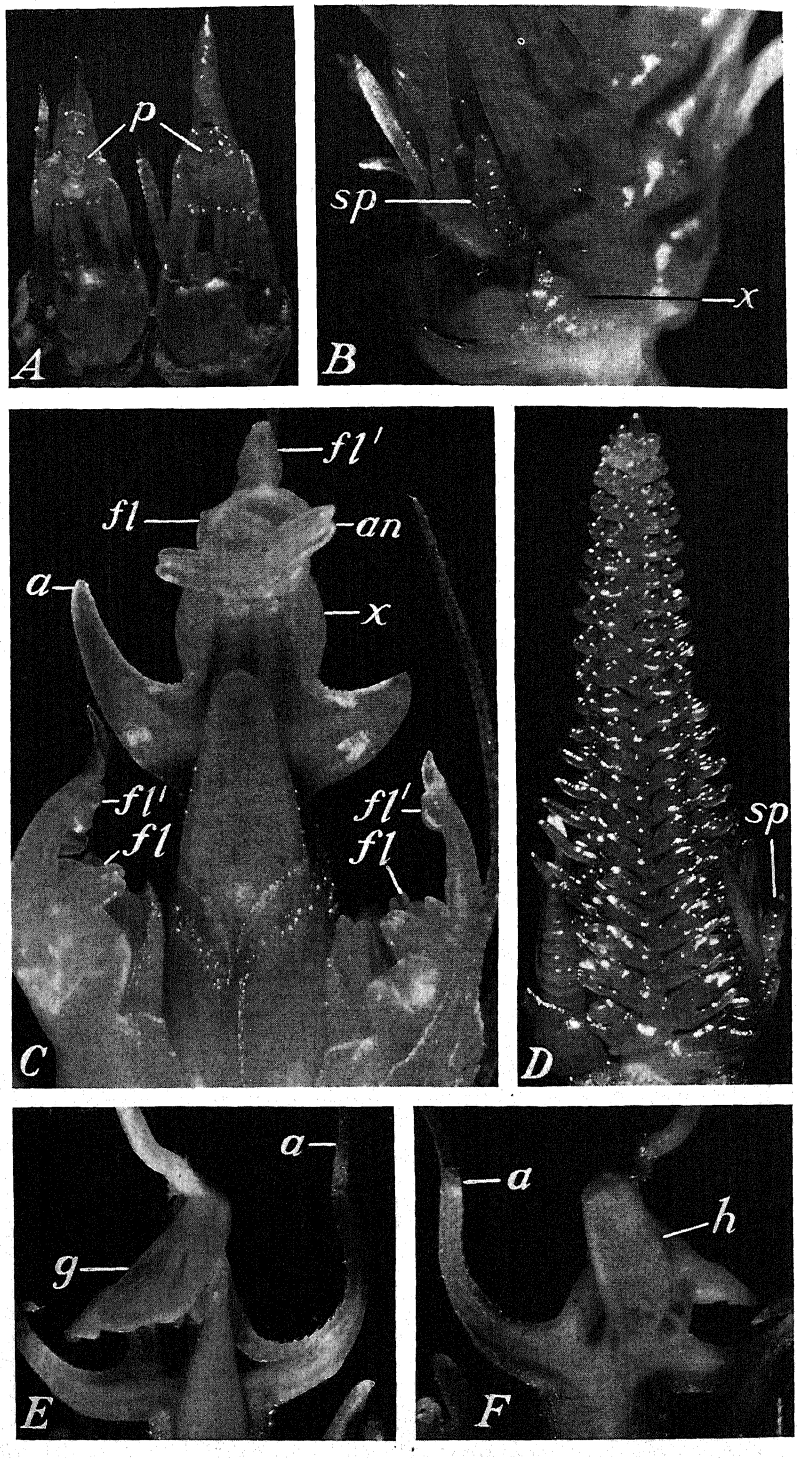
When the tip of the lemma of the hooded variety has elongated to the point where it begins to resemble an awn (pl. 1, *C* and *D*) the hood primordia begin to appear (pl. 1, *C*, *x*). From this point, the development of the lemmas of hooded and awned barley is different.

EXPLANATORY LEGEND FOR PLATE 1

A, An early stage in the development of a spike of Nepal barley: *p*, Pistil initial; *an*, anther initial; *g*, lemma initial; *s*, spikelet initial; *t*, undifferentiated tip of the spike. × 25. *B*, A spike of a six-row, awned barley at about the same stage of development as that shown in plate 1, *A*: *e*, Empty glume initial. × 25. *C*, Part of a spike of Nepal barley at the beginning of hood differentiation: *x*, A spikelet with an accessory flower developing. × 20. *D*, Part of a spike of awned barley at about the same stage as that in plate 1, *C*, showing the beginning of the growth of awns. × 25. *E*, An adaxial view of the beginning of the differentiation of the accessory flower initial: *an*, Anther of the normally placed flower; *p*, pistil initial of the normally placed flower; *fl*, accessory flower initial. × 25. *F*, Beginning of the differentiation of the palea of the accessory flower: *g*, Palea initial. × 25. *G*, Anther differentiation of the accessory flower: *an*, Anther initial. × 25. *H*, Two accessory flowers side by side; four anthers and the pistil initial are clearly shown in the flower at the left: *x*, Accessory flower of the side spikelet; *an*, anther; *p*, pistil initial; *fl'*, second accessory flower. × 25. *I*, A more advanced stage of the development of the anthers and pistil of the accessory flower: *an*, Anthers; *p*, pistil initial. × 25. *J*, An accessory flower showing four anthers and pistil initial: *an*, Anther; *p*, pistil initial; *fl'*, second accessory flower. × 25. *K*, *L*, and *M*, An abaxial view of the lemmas of the normally placed flowers showing successive stages of hood development: *a*, Laterally placed awnlike appendage of the hood. × 25.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

The hood primordium first appears as a dome-shaped outgrowth on the adaxial side and near the tip of the lemma (pl. 1, *E*, *fl*), and from this primordium the parts of the accessory flower differentiate. Unlike normal flowers, there is no indication of a leaf primordium subtending the accessory flower of the hood.

When the hood primordium is first seen the flower parts of the normally placed flower are not very far advanced in development. The anthers are short and show the development of the locules (pl. 1, *E*, *an*), but the pistil is not far enough advanced to show any development of the styles (pl. 1, *E*, *p*).

Differentiation of the flower parts of the accessory flower in the hood, with but one exception, follows the same sequence as those of the normally placed flower. In the normally placed flower the first flower part to differentiate is the lemma (pl. 1, *A*, *g*), but, since the tip of the lemma of the normally placed flower serves as the lemma of the accessory flower, no lemma is produced for the accessory flower. The first flower part to differentiate in the accessory flower is the palea (pl. 1, *F*, *g*).

It should also be pointed out that the accessory flower is not subtended by empty glumes as is the case with the flower of the normal spikelet in plate 1, *B*, *e*. Since there are no empty glumes present, the floral part of the hood cannot be classified as a spikelet, but must be considered as a flower.

Soon after the palea of the accessory flower differentiates, the anthers and pistil differentiate just as in normally placed flowers. The successive steps in the anther differentiation are shown in plate 1, *G*, *an;* *H*, *an;* *I*, *an;* and *J*, *an;* and in plate 2, *C*, *an*. Successive stages of pistil development are shown in plate 1, *H*, *p;* *I*, *p;* and *J*, *p*. While functional pistils do not often develop in the flower of the hood, the pistil primordia are present and go through a part of their developmental cycle.

The description of accessory flower development has been for the central spikelets only. Hoods and their accessory flowers also develop on the lemmas of the side spikelets (pls. 1, *H*, *x*, and 2, *C*, *fl* and *fl′*). No hoods developed on the empty glumes of the plants grown for this study, but Harlan (*4*) has found them on the empty glumes of the progeny of certain crosses.

More than one flower may develop on the lemma. They may be located side by side (pl.1, *H*, *fl′*) or above each other (pls. 1, J, *fl′* and 2, *C*, *fl′*). The more basal flower is the one which attains the greatest development.

Only three anthers develop in a normal flower and this is the usual number in the accessory flowers. Three cases are shown (pls. 1, *H* and *J*, and 2, *A*) where four anthers have developed.

Pistils begin development in the supernumerary flowers of the side spikelets as well as in the central spikelets (pl. 2, *A*, *p*). This obser-

EXPLANATORY LEGEND FOR PLATE 2

A, Accessory flowers of the side spikelets showing the differentiation of pistils: *p*, Pistil initials. × 25. *B*, Base of a barley spike showing two supernumerary spikes: *sp*, Supernumerary spike at the position of the central spikelet; *x*, supernumerary spike at the position of the side spikelet. × 15. *C*, Spikelets of barley showing accessory flowers: *fl*, First-formed accessory flowers; *fl′*, second-formed accessory flowers; *a*, awnlike appendage of the hood; *x*, hood; *an*, anther. × 17. *D*, A spike of barley showing supernumerary spikes (*sp*) on both sides at the base. × 20. *E*, An adaxial view of the accessory flower nearing complete development: *g*, Palea; *a*, lateral awnlike appendage of the hood. × 8. *F*, An adaxial view of the hood: Awnlike appendage of the hood; *h*, hood. × 8.

vation is contrary to a statement made by Arber (*1*) that the accessory flowers of the side spikelets are male and only those of the central spikelets are complete.

Several stages in the development of the lemma into a structure resembling a hood are shown. In the earlier stages (pl. 1, *K*) the abaxial side of the lemma resembles that of an awned variety except that the margins of the lemma protrude slightly at a point opposite the point of origin of the accessory flower.

The margins of the lemma continue to grow (pl. 1, *L*, *a*, and *M*, *a*) and form a pair of awnlike points (pl. 2, *C*, *a*, *E*, *a*, and *F*, *a*). That portion of the lemma between the awnlike lateral projections continues to grow (pl. 2, *C*, *x*) and serves as the lemma of the accessory flower. As maturity approaches the tip of the lemma has the appearance of a hood (pl. 2, *F*, *h*) with two lateral awnlike appendages (pl. 2, *E*, *a*, and *F*, *a*).

DEVELOPMENT OF SUPERNUMERARY SPIKES

Supernumerary spikes, in those barley varieties studied, differentiate at the basal nodes of the main spike (pl. 2, *B*, *sp*, and *D*, *sp*). They arise most frequently in the axil of the lemma of the central spikelets, but they may appear at the position of the side spikelets (pl. 2, *B*, *x*).

A description of the way the spikes shown in figure 1 are joined may be of interest. Both the primary spike (fig. 1, *sp*) and the largest supernumerary spike (fig. 1, *sp'*) arise at the same node. At this node there is a normal leaf (fig. 1, 2, *l*). The spike (fig. 1, *sp'*) is attached to a culm consisting of three nodes. At the basal node the leaf is not normal but is biparted, one division of which resembles a prophyllum (fig. 1, *x*). Normal leaves are found at both the nodes above the basal one of the spike in figure 1, *sp'*.

The smallest supernumerary spike (fig. 1, *sp''*) originates from the primary spike at the point usually occupied by the central spikelet (pl. 2, *B*, *sp*). There is no indication of a prophyllum, but the empty glumes are present. On both sides of the smaller supernumerary spike (fig. 1, *sp''*) were found the rudiments of spikes subtended by the empty glumes of the side spikelets.

The culm beneath the primary spike (fig. 1, *sp*) is flattened and has three elongated internodes. The first internode of the primary spike has also elongated. No leaves were found at any of these nodes. The smallest supernumerary spike (fig. 1, *sp''*) is attached at the second node.

DISCUSSION

There has been some difference in the opinions expressed by various workers regarding the number of pairs of genes involved in the development of the hooded character. The majority of the investigators cited by Matsuura (*7*) have interpreted their results upon a monogenic basis, but others have said that two or more pairs of genes are involved. Regardless of the number of gene pairs involved, it may be of interest to discuss briefly what it seems would be necessary for the gene to do to produce the hooded character.

The description of the development of the accessory flower shows that it differs from the normally placed flower in its point of origin and in its pattern of development. The accessory flower originates as an outgrowth from the ventral surface of the lemma, a modified leaf,

rather than from an axis. A normally placed flower has two flowering glumes, a lemma and a palea, but the accessory flower has only a palea since the tip of the lemma of the normally placed flower serves as the lemma of the accessory flower. Finally, at the point where the accessory flower differentiates, the lemma of the normally placed flower develops into a hoodlike structure with two lateral awnlike appendages.

If the pattern of development of the accessory flower were exactly like that of a normally placed flower, the gene action in the production of hoods could be rather easily explained. Every cell is believed to possess the same gene content as the fertilized egg, and since normal barley plants have the ability to produce floral centers, all that the genes for hooded would need to do would be to initiate at the proper time, and at the proper point on the developing lemma, the production of a floral center. But, as has been pointed out, the accessory flower does not follow, exactly, the same pattern of development as the normally placed flower.

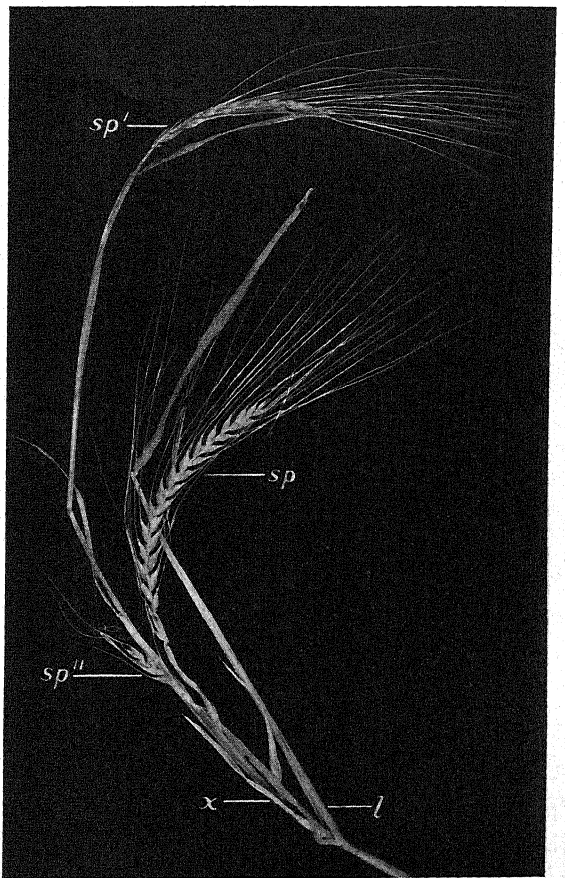


FIGURE 1.—The primary spike and supernumerary spikes of barley at the mature stage: *l*, Normal leaf; *x*, a biparted leaf; *sp''*, supernumerary spike originating at the second node of the primary spike; *sp*, primary spike; *sp'*, supernumerary spike originating at the same node as the primary spike.

If a single gene or gene pair were responsible for the entire pattern of hood development, it would have to initiate the development of a floral center, inhibit the development of a lemma, and initiate the development of the hood and its appendages. It is not consistent with the accepted ideas of gene action to suppose that a single gene or gene pair both initiates and inhibits. Therefore, in view of the statement just made, a simple explanation would be that there is a group of closely linked genes responsible for the production of the hooded character. The first gene to act is the one which initiates the development of the floral center. Following the development of the floral center, the gene or genes inhibiting the development of the lemma act, and then those which control the development of the hood and its

appendages would produce their effect. Such an explanation is in accordance with the accepted ideas of gene action.

However, regardless of the correctness of the above explanation, there is another point for consideration. While the explanation of the inheritance of the hooded character on a monogenic basis accounts satisfactorily for the ratio of the phenotypes in the F_2, the explanation does not fit so well in accounting for the morphological development of the character. It seems reasonable to require that the genetic assumptions account not only for the F_2 ratio but for the developmental processes as well.

Other deviations from the normal in the development of the accessory flower, such as more than one floral center on the lemma, four anthers instead of three, restricted pistil and awn development, need not be discussed. It is believed that these differences could be explained without the necessity of assuming the action of special genes.

The development of supernumerary spikes in the awned barley varieties previously mentioned is explained as the result of the action of environmental factors rather than as the result of the operation of special genes. During the period from December 19 to February 22 when the barley spike was differentiating the temperatures were relatively low. The average maximum was 68.1° F., the average minimum 53.2°, and the average mean 60.8°. This period of time also extended through the short days of the year. It is believed that the low temperature and short days were principally responsible for the production of the supernumerary spikes. Such an assumption is in accordance with the results of the investigation of Hurd-Karrer (*6*) with Turkey wheat. With an 8-hour day and low temperatures she found that Turkey wheat developed a branched head.

SUMMARY

The morphological changes which occur in the development of the hood in barley were studied by dissecting spikes at successive stages of development. Photomicrographs of the principal stages of development are shown.

Hood primordia first appear as dome-shaped outgrowths on the adaxial side, near the tip of the lemma, and from these primordia the parts of the accessory flower differentiate.

A lemma is not produced in the differentiation of the accessory flower since the tip of the lemma of the normally placed flower serves as its lemma. The first flower part to differentiate is the palea, followed in turn by the anthers and pistil as in normally placed flowers.

More than one floral center may develop. They may be placed one above the other or side by side.

Although anthers and a pistil of the accessory flower may begin development, they usually are nonfunctional.

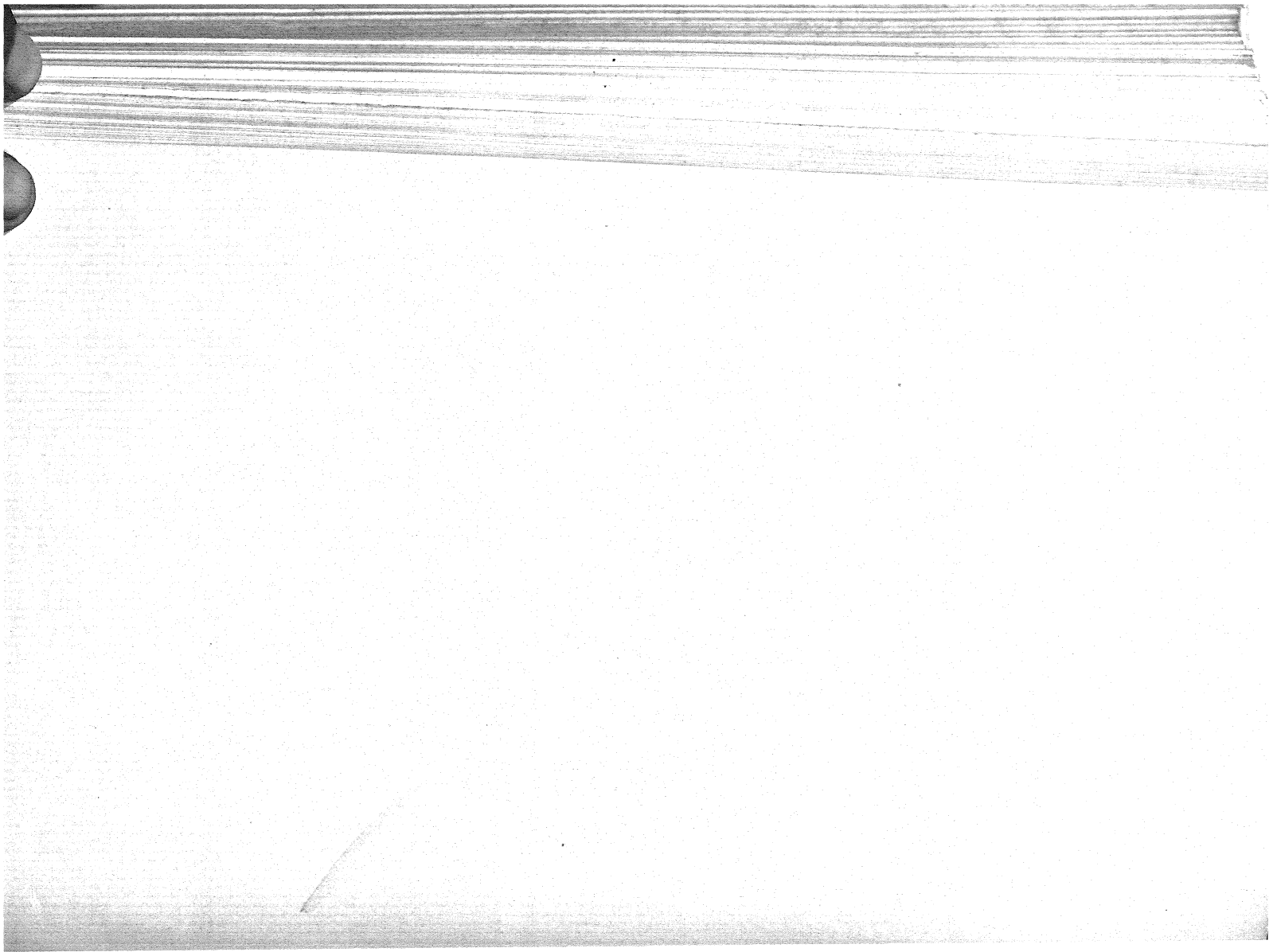
The tip of the lemma of the normally placed flower develops into a structure resembling a hood, and from the lateral margins two awn-like appendages develop. While an awn begins to develop at the tip of the lemma, its growth is suppressed by the growth of the accessory flower.

Supernumerary spikes were found to arise from the base of the primary spike. They probably resulted from the effect of a combination of low temperature and a short day.

Although most investigators explain the inheritance of hoods in barley on a monogenic basis, such an explanation is not entirely satisfactory when an attempt is made to explain how a single gene or gene pair could bring about the morphological changes necessary to produce the hooded character.

LITERATURE CITED

(1) ARBER, AGNES.
1934. THE GRAMINEAE: A STUDY OF CEREAL, BAMBOO, AND GRASS. 480 pp., illus. Cambridge.
(2) BIFFEN, ROWLAND H.
1905. THE INHERITANCE OF STERILITY IN THE BARLEYS. Jour. Agr. Sci. [England] 1: 250–257, illus.
(3) BONNETT, O. T.
1935. THE DEVELOPMENT OF THE BARLEY SPIKE. Jour. Agr. Research 51: 451–457, illus.
(4) HARLAN, HARRY V.
1931. THE ORIGIN OF HOODED BARLEY. Jour. Heredity 22: 265–272, illus.
(5) HOR, KWEN SHEN.
1924. INTERRELATIONS OF GENETIC FACTORS IN BARLEY. Genetics 9: 151–180.
(6) HURD-KARRER, ANNIE M.
1933. COMPARATIVE RESPONSES OF A SPRING AND A WINTER WHEAT TO DAY LENGTH AND TEMPERATURE. Jour. Agr. Research 46: 867–888, illus.
(7) MATSUURA, HAJIME.
1933. A BIOGRAPHICAL MONOGRAPH ON PLANT GENETICS (GENIC ANALYSIS) 1900–1929. Ed. 2, rev. and enl., 787 pp. Sapporo.
(8) ROBERTSON, DAVID WIELD.
1929. LINKAGE STUDIES IN BARLEY. Genetics 14: 1–36, illus.



INFLUENCE OF JAPANESE BEETLE INSTAR ON THE SEX AND POPULATION OF THE PARASITE TIPHIA POPILLIAVORA[1]

By M. H. BRUNSON[2]

Assistant entomologist, Division of Fruit Insect Investigations, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture

INTRODUCTION

Tiphia popilliavora Roh. was first shipped to this country from Japan in 1920 as part of the program to introduce parasites that would reduce the population of the Japanese beetle (*Popillia japonica* Newm.). Since that time 557 colonies of this parasite, each consisting of 100 females, have been liberated in New Jersey, Pennsylvania, Delaware, and Maryland, 553 of which were derived from 4 colonies established from the imported material. In the course of scouting 194 of these colonies for adults over a period of years, the potential effectiveness of the parasite in reducing Japanese beetle populations was found to vary, not only at different recovered colonies each year, but at all colonies at intervals of several years. An investigation was therefore undertaken to learn the cause of such variations. The data were obtained at Moorestown, N. J., and vicinity from 1931 to 1935. A preliminary report regarding the probable factors that cause a change in the population of *T. popilliavora* was published in 1934.[3]

REVIEW OF LITERATURE

That certain insects are able to control the sex of their progeny has long been known, but only a few studies that clearly demonstrate this phenomenon have been reported. Chewyreuv[4] found that when *Pimpla instigator* F. parasitized pupae of different sizes a predominance of males emerged from the smaller pupae and a predominance of females from the larger pupae. When small, intermediate, and large pupae were furnished the female parasite, a majority of males issued from both the small and the intermediate pupae and a majority of females issued from the large pupae. When only the intermediate and the small pupae were furnished the female parasite, females predominated among the parasites emerging from the intermediate pupae and males among those emerging from the small pupae. Holdaway[5] found that when *Alysia manducator* Pantzer parasitized host larvae that produced small pupae the parasite progeny were largely males, and from hosts that formed large pupae parasites of both sexes, but more females than males, were produced. Seyrig[6] obtained

[1] Received for publication April 27, 1938; issued September 1938.

[2] The author is indebted to J. L. King, in charge of parasite investigations at the Japanese beetle laboratory, Moorestown, N. J., for helpful suggestions and criticisms in the course of this study.

[3] BRUNSON, M. H. THE FLUCTUATION OF THE POPULATION OF TIPHIA POPILLIAVORA ROHWER IN THE FIELD AND ITS POSSIBLE CAUSES. Jour. Econ. Ent. 27: 514–518. 1934.

[4] CHEWYREUV, IV. LE RÔLE DES FEMELLES DANS LA DÉTERMINATION DU SEXE DE LEUR DESCENDANCE DANS LE GROUPE DES ICHNEUMONIDES. Compt. Rend. Soc. Biol. [Paris] 74: 695–699. 1913.

[5] HOLDAWAY, F. G., and SMITH, H. FAIRFIELD. A RELATION BETWEEN SIZE OF HOST PUPARIA AND SEX RATIO OF ALYSIA MANDUCATOR PANTZER. Aust. Jour. Expt. Biol. and Med. Sci. 10: 247–259, illus. 1932.

[6] SEYRIG, ANDRÉ. RELATIONS ENTRE LE SEXE DE CERTAINS ICHNEUMONIDES (HYM.) ET L'HÔTE AUX DÉPENS DUQUEL ILS ONTE VÉCU. Bull. Soc. Ent. France 40: 67–70. 1935.

similar results with *Echthromorpha hyalina* Sauss. and *Pimpla maculiscaposa* Seyrig reared from pupae of several species of Lepidoptera. He attributed this difference in sex to the infertility of small females, which did not attract males so readily as did the large females, and to the small females selecting the small pupae and the large females the large pupae for oviposition.

LIFE CYCLE OF PARASITE AND HOST

There is only one generation of *Tiphia popilliavora* annually. Adults appear in the field for the most part between August 5 and 31. Females are most abundant between August 10 and 28. Species of *Tiphia* develop as external parasites on the host. The egg is placed ventrally on the host larva, usually between the fifth and sixth abdominal segments, and hatches within 4 or 5 days at 75° F. At this temperature the larval period lasts from 10 to 12 days, and then the cocoon is formed in a small cell which the host larva has made in the ground at a depth of about 5 inches. Shortly after spinning the cocoon the larva changes into a prepupa, and in this stage it hibernates. The pupa is formed the following July, and the adult emerges in August.

The Japanese beetle also has only one generation a year. There are three larval instars, and the second and the third instars are accepted by the female *Tiphia* for oviposition. The insect hibernates as a third-instar larva and pupates late in the spring. Adults begin to appear in June and may be found in the field through August.

REARING METHODS

Most of the female parasites used in the experiments were field-collected, although some were reared and mated in the insectary. Host larvae were obtained in the field daily except Sunday, and were used the following day. The containers for confining the parasites and host larvae for parasitization were 6-ounce tin salve boxes. Except when otherwise noted, four host larvae were placed in each container daily. To prevent mortality due to fighting, they were restricted to individual compartments made of tin. Soil was then introduced and packed to within a half inch of the top. Water and food for the parasite, the food consisting of a thick paste of powdered sugar and honey, were placed on small pieces of waxed paper on the soil. The female parasite was then introduced and the lid put on the container. The containers were kept for 24 hours in a cellar maintained at 74° F. At the end of this time the parasite was transferred to another container with a fresh supply of host larvae, food, and water, and the parasitized hosts were placed in pans 18 inches square and 2 inches high, each divided by wooden separators into 196 compartments 1 inch square. One parasitized larvae and a few grains of wheat were placed in each compartment, which was then packed with soil. The pans were also kept in a cellar at 74° and after 21 days were examined for parasite cocoons. The cocoons were placed individually in 2-dram homeopathic vials and stored in trays for overwintering and emergence the following year.

INSTAR OF HOST LARVAE PREFERRED FOR OVIPOSITION

Observations on the response of *Tiphia popilliavora* to host larvae of different instars showed that both second-instar and third-instar larvae are accepted for oviposition, although third-instar larvae, which are about twice the size of second-instar larvae, apparently are preferred. To determine the degree of preference shown for third-instar larvae, females were placed in containers in which larvae of the two instars were made available either together or separately. In 1932 both a second-instar and a third-instar larva were placed in each of four compartments in propagation containers. In 1933 one host larva was placed in each of eight compartments, a larva of the second instar being alternated with one of the third instar. The results of these observations are shown in table 1.

TABLE 1.—*Instar of host larvae preferred by female parasites for oviposition*

Year	Female parasites	Third-instar hosts parasitized	Second-instar hosts parasitized	Ratio of third-instar to second-instar hosts parasitized
	Number	*Number*	*Number*	
1932	26	991	187	5.3:1
1933	29	348	20	17.4:1

RELATION BETWEEN INSTAR OF HOST AND SEX OF PARASITE PROGENY

PARASITES FURNISHED HOSTS OF ONE INSTAR ALONE AND OF BOTH INSTARS TOGETHER

From 1931 to 1934, inclusive, observations were made on the relation between the size or the instar of the host larvae and the sex of the parasite progeny. In 1931 and 1932 adult emergence was from cocoons formed when the female parasites were furnished only second-instar larvae for parasitization; in 1933 both second-instar and third-instar host larvae, and in 1934 only third-instar larvae, were provided. The results are summarized in table 2.

TABLE 2.—*Sex ratio of parasite progeny emerging from cocoons formed on second-instar and third-instar host larvae*

Year	Female parasites	Hosts	Cocoons	Hosts on which cocoons were formed	Emergence of parasite progeny		
					Total	Males	Females
SECOND-INSTAR HOST LARVAE							
	Number	*Number*	*Number*	*Percent*	*Number*	*Percent*	*Percent*
1931	184	2,510	1,314	52.4	863	96.4	3.6
1932	120	3,107	1,232	39.7	694	89.5	10.5
1933	26	316	152	48.1	117	97.4	2.6
Total	330	5,933	2,698	45.5	1,674	93.6	6.4
THIRD-INSTAR HOST LARVAE							
1933	26	1,050	578	55.0	401	32.9	67.1
1934	29	350	204	58.3	150	32.7	67.3
Total	55	1,400	782	55.8	551	32.8	67.2

Emergence of adult parasites from both large and small second-instar host larvae, obtained in the 1931 and 1932 experiments, showed conclusively that the sex ratio of parasite progeny developing on larvae of this instar is the same regardless of the size of the host; consequently, the data are not included in this paper.

The adults that emerged from cocoons formed on second-instar larvae were predominantly males, while those obtained from parasitized third-instar larvae were largely females. Regardless of whether both second-instar and third-instar larvae, second-instar larvae alone, or third-instar larvae alone are furnished females, the sex ratio of the parasite progeny is practically constant for each instar of the host. There was a difference of 10.3 in the percentage of parasitized host larvae of the two instars that yielded cocoons. The difference may be explained by the fact that many of the second-instar larvae molt before the parasite egg has hatched, and the parasite egg is dislodged along with the larval skin.

PARASITES FURNISHED HOSTS IN THE SECOND INSTAR AND THE THIRD INSTAR THE SAME DAY AND ON ALTERNATE DAYS

In 1934 two series of females reared and mated in the insectary were used to ascertain whether the sex ratio of the parasite progeny when the female parasites were furnished host larvae in the second instar and the third instar on alternate days would differ from the sex ratio when they were furnished host larvae of both instars on the same day. The results are shown in table 3. It will be seen that the parasite progeny emerging from second-instar hosts are largely males and those from third-instar hosts are predominantly females.

TABLE 3.—*Sex ratio of parasite progeny when females were furnished second-instar and third-instar hosts on alternate days and when furnished hosts of both instars the same day*

Females (number)	Instar of host larvae	Cocoons	Emergence of parasites		
			Total	Males	Females
DIFFERENT INSTARS ON ALTERNATE DAYS					
		Number	*Number*	*Percent*	*Percent*
93	Third	693	517	40.2	59.8
	Second	169	118	99.2	.8
BOTH INSTARS ON SAME DAY					
30	Third	585	424	46.2	53.8
	Second	338	226	100.0	0

PARASITE EGGS TRANSFERRED FROM HOSTS OF ONE INSTAR TO THOSE OF ANOTHER

Second-instar and third-instar larvae that had been parasitized by females reared and mated in the insectary were obtained, and the parasite eggs from each host instar were transferred to the other instar.

The transfer of eggs from one instar to another presented a special problem. Owing to the activity of the host larva and its smooth outer body wall, it was difficult to find an adhesive that would attach the parasite egg securely to the host. Wood glue, shellac, rosin dissolved

in alcohol, celluloid dissolved in acetone, egg albumen, and several commercial adhesives were tried, but the best results were obtained with a mixture of tire-patch cement and benzine. The eggs were removed from the host larvae and placed on moist blotting paper in Petri dishes. The larvae were then placed in a jar with a few drops of ether to quiet them. Eggs from host larvae of one instar were attached to host larvae of the other instar in the position of the original egg, care being taken to place the ventral side of the egg in contact with the host and the anterior pole of the egg along the median ventral line. The host larvae were placed in flats and handled as previously described for the formation of cocoons.

The data given in table 4 show that the sex of the parasite progeny is determined at the time of oviposition, and is not affected by the quantity or quality of food available for development.

TABLE 4.—*Sex ratio of parasites emerging from cocoons formed when parasite eggs were transferred from second-instar to third-instar host larvae and vice versa*

Eggs transferred from—	Total hosts parasitized	Total cocoons	Parasites emerged		
			Total	Males	Females
	Number	*Number*	*Number*	*Percent*	*Percent*
Second-instar to third-instar host	700	125	39	97.4	2.5
Third-instar to second-instar host	574	89	34	44.1	55.8
Check (third-instar host larvae)	925	580	334	38.3	61.6

SEX OF PARASITE PROGENY RESULTING FROM PARASITIZATION OF HOST LARVAE BROUGHT FROM THE FIELD IN AUGUST AND SEPTEMBER

To learn the effect of the larval instar of the Japanese beetle in the field on the sex ratio of *Tiphia popilliavora* parasites, in 1933 host larvae were brought from the field daily in August and September and submitted to parasitization by females reared and mated in the insectary. The emergence data are summarized in table 5. That the data might be compared with average conditions in the field during this period, the ratios of second-instar to third-instar hosts as determined from surveys made in 1932, 1933, and 1934 are included.

TABLE 5.—*Parasite emergence from cocoons formed on hosts taken from the field in August and September 1933, and average percentage of hosts in second and third instars in 1932, 1933, and 1934*

Period	Cocoons, 1933	Parasites emerged, 1934			Average hosts in indicated instar, 1932–34	
		Total	Males	Females	Second	Third
	Number	*Number*	*Percent*	*Percent*	*Percent*	*Percent*
Aug. 11–20	607	290	94.5	5.5	67.2	1.5
Aug. 21–31	1,578	847	81.5	18.5	61.4	24.6
Sept. 1–10	1,917	1,312	45.4	54.7	42.3	51.2
Sept. 11–20	799	491	34.0	66.0	26.5	72.5

EFFECT ON PARASITE POPULATION OF HOST ABUNDANCE AND INSTARS AT DIFFERENT COLONY POINTS DURING AUGUST

In August 1935 three surveys were made to determine whether the abundance of parasites at some colony points and a scarcity or absence at others could be attributed to a difference in the relative number of host larvae in the third instar or in the total population of host larvae. Seven colony points were selected to represent various degrees of abundance of the parasite.

The results of these surveys are shown in table 6. The highest percentage of third-instar hosts and also the greatest number of host larvae per square foot occurred at Lansdowne and Llanerch, Pa., the colony points where the parasite has appeared in greatest abundance. Host larvae were fairly abundant at Langhorne, Pa., but the percentage of larvae in the third instar was not sufficiently high for the parasite to be abundant. The number of host larvae per square foot at Lippincott's pasture was sufficient to maintain a colony, but the percentage in the third instar was very low. The primary reason for the scarcity of parasites at Herker's pasture was the small population of the host larvae, for the percentage of third-instar larvae was as high as at Lansdowne. At Indian Hill and Camp Dix neither the total host population nor the percentage of hosts in the third instar was high enough to maintain a colony.

TABLE 6.—*Results of survey of host larvae at various colony points in August 1935*

Colony point	Abundance of parasites	Date of survey	Diggings	Total hosts found	Average hosts per square foot	Hosts in indicated instar	
						Second	Third
			Number	*Number*	*Number*	*Percent*	*Percent*
Indian Hill pasture, Jobstown, N. J.	Absent	Aug. 3	84	230	2.7	6.9	0
		14	84	142	1.7	54.9	0
Camp Dix, Wrightstown, N. J.	do	23	84	245	2.9	79.2	4.0
		5	82	263	3.2	16.2	0
Herker's pasture, Moorestown, N. J.	Scarce	15	82	293	3.6	49.4	5.8
		26	82	209	2.5	88.5	4.3
Lippincott's pasture, Jobstown, N. J.	do	1	33	66	2.0	3.0	0
		12	46	80	1.7	33.7	1.2
		21	46	73	1.6	56.1	12.3
Langhorne Country Club, Langhorne, Pa.	Fairly abundant.	2	82	403	4.9	.7	0
		13	82	626	7.6	44.0	0
		22	82	739	9.0	87.6	.5
Lansdowne Country Club, Lansdowne, Pa.	Very abundant.	16	62	683	11.0	55.5	.4
		27	62	539	8.7	73.1	1.6
		8	82	647	7.9	35.5	0
Llanerch Country Club, Llanerch, Pa.	do	19	67	787	11.7	64.6	1.1
		28	82	821	10.0	69.4	12.3
		9	56	1,198	21.4	57.7	0
		20	75	1,066	14.2	79.6	1.9
		29	75	934	12.5	66.5	23.9

PERIODIC FLUCTUATION IN RELATIVE NUMBER OF THIRD-INSTAR HOSTS AND ITS POSSIBLE EFFECT ON PARASITE POPULATION

At intervals of several years the population of the parasite has suffered a general reduction at all colony points regardless of the population of host larvae in the soil. The discovery of the reaction of female parasites to second-instar and third-instar hosts available for parasitization and the resultant sex ratio of the parasite progeny suggested that a scarcity of third-instar larvae throughout the area in which parasites had been distributed might explain the periodic fluctuation of the parasite population.

A survey was conducted at eight points in New Jersey and Pennsylvania from 1932 to 1935, inclusive, to ascertain the relative number of the various immature stages of the Japanese beetle present in the field during 10- or 11-day periods in July, August, and September. The results of this survey for August, the period in which *Tiphia popilliavora* adults appear in the field in greatest abundance, are shown in table 7.

TABLE 7.—*Relative abundance of the immature stages of the Japanese beetle in the field in August 1932, 1933, 1934, and 1935*

Year	Period of survey	Eggs	First instar	Second instar	Third instar
		Percent	*Percent*	*Percent*	*Percent*
1932	Aug. 1–10	20.1	40.4	39.4	0.1
	11–20	5.5	17.4	75.4	1.7
	21–31	.04	5.2	66.3	28.4
1933	1–10	30.6	38.5	30.8	.1
	11–20	5.1	37.7	56.0	1.1
	21–31	1.3	23.4	50.4	24.9
1934	1–10	21.9	43.8	33.5	.8
	11–20	5.3	22.4	70.7	1.6
	21–31	.8	11.1	67.4	20.7
1935	1–10	28.5	59.8	11.7	.0
	11–20	11.5	48.9	39.6	.0
	21–31	1.7	31.5	64.6	2.2

DISCUSSION

The data obtained in the course of this study show that the variation of *Tiphia popilliavora* populations at different colonies annually, and at all colonies at intervals of several years, is a result of the reaction of the parasite to Japanese beetle larvae of different instars on which oviposition occurs, and to variations in the total population of the host in the soil at the time adult parasites appear.

During August, the period that adult parasites are most abundant, the three larval instars of the host are available for parasitization (table 7), but only larvae in the second and third instars are selected for oviposition. A definite preference was shown by the parasite for third-instar larvae for oviposition (table 1), although second-instar larvae were readily accepted (tables 2 and 3). Regardless of whether both second-instar and third-instar larvae, second-instar larvae alone, third-instar larvae alone (table 2), or larvae of each instar on alternate days (table 3) were furnished parasites for oviposition, the progeny from second-instar larvae were largely males while those from third-instar larvae were chiefly females. When parasite eggs were transferred from second-instar to third-instar larvae, and vice versa, the sex ratio of the progeny was not changed (table 4). These data show that the sex of the progeny is determined at the time the egg is placed on the host larva, and is not affected by the quantity or quality of the food available for development of the parasite.

That the sex ratio of the parasite progeny in the field might vary with the proportion of larvae in the second and third instars is shown in table 5. There is a definite relation between the increase in the proportion of larvae in the third instar at intervals of 10 to 11 days and the increase in female parasite progeny.

These facts explain the variation in the parasite population at different colonies annually where the third-instar larvae might be relatively abundant or scarce regardless of the total larval population

(table 6), and at all colonies at intervals of several years when a variation in the relative proportion of third-instar to second-instar larvae might result in a scarcity of third-instar larvae although the total larval population may be normal (table 7).

SUMMARY

A study was conducted from 1931 to 1935 on the relation between *Tiphia popilliavora* Roh. and the three larval instars of its host, the Japanese beetle (*Popillia japonica* Newm.), for the purpose of explaining the variation in the parasite population at different colony points annually and at all colony points at intervals of several years.

Both second-instar and third-instar host larvae were accepted for parasitization, although third-instar larvae were preferred. Parasite development went to completion on both second-instar and third-instar larvae.

Parasites from second-instar larvae were predominantly males and those from third-instar larvae were predominantly females.

Parasite eggs transferred from second-instar to third-instar larvae produced a preponderance of males, and parasite eggs transferred from third-instar to second-instar larvae produced both males and females but more females than males, indicating that the sex of the progeny is determined at the time the egg is placed on the host.

The prevailing host stage in the field during August, the period of greatest adult parasite activity in the field, is the second instar, although third-instar larvae usually occur in considerable numbers the latter part of the month. The scarcity of third-instar host larvae during the period of greatest parasite activity greatly reduces the effectiveness of the parasite regardless of the total population of the host larvae.

The variation in the relative number of host larvae that are in the third instar and also in the total host population at different colony points in the area heavily infested with the Japanese beetle tends to cause a variation in the parasite population at different colony points. A gradual or marked decrease in the number of host larvae in the third instar at all colony points at intervals of several years explains the marked reduction in the parasite population at similar intervals.

MICROCHEMICAL STUDIES OF POTATO TUBERS AFFECTED WITH BLUE STEM DISEASE [1]

By L. M. HILL, *assistant in plant pathology, Department of Plant Pathology and Forestry,* and C. R. ORTON, *director, West Virginia Agricultural Experiment Station* [2]

INTRODUCTION

A new disease of potato (*Solanum tuberosum* L.) in West Virginia was described by Orton and Hill (*6*) [3] in 1937. It has become known as blue stem because of the characteristic discoloration of the stem during the later stages of the disease.

This paper is devoted to comparative microchemical studies of healthy and diseased tubers of the Russet Rural variety. Such studies present certain complicated factors because the test for any specific compound may be profoundly modified by the presence of other substances that mask or interfere with the tests.

The term "necrotic area" refers to the area composed of apparently dead cells as shown by a brown discoloration, which is usually accompanied by a granular deposit. The term "zone" applies to an area of definite extent surrounding the necrotic region.

The technique used was that of Tunmann (*7*), Molisch (*5*), Emich (*3*), Hinrichs (*4*), and Chamot (*1*). The use of the petrographical microscope is described by Chamot and Mason (*2*).

MEMBRANE SUBSTANCES

Cellulose.—Place sections in a drop of iodine-potassium iodide; add a drop of 75 percent sulphuric acid under the cover glass. Cellulose membranes become blue. Cellulose is birefringent. Cellulose tests applied directly to the necrotic areas in the phloem and parenchyma proved negative (pl. 1, *A* and *B*). Schultze's reagent dissolved the suberized deposit resulting from the disease; when this treatment was followed by the polarized light test, the results were positive (pl. 2, *A* and *B*). The zones gave positive tests throughout. When the tests were applied directly to the necrotic trachea without the use of Schultze's reagent, the tests were positive (pl. 3, *C* and *D*).

The cell walls remaining after this treatment were hydrolyzed in 75 percent sulphuric acid. The fact that the dissolution of the deposited substances in the necrotic areas leaves the cell walls intact suggests that these substances are formed from the cytoplasm rather than from the cellulose walls.

Pectic Substances.—Treat with a dilute solution of ruthenium red for 20 minutes, wash thoroughly. All pectic substances stain red; they are soluble in 2 percent potassium hydroxide. With 3 percent ammonium oxalate they give calcium oxalate crystals. Tissues treated

[1] Received for publication February 2, 1938; issued September 1938. Scientific Paper No. 201 of the West Virginia Agricultural Experiment Station.

[2] The authors are greatly indebted to Dr. S. H. Eckerson for her aid in microchemical technique and for many valuable suggestions, and also to the Boyce Thompson Institute for Plant Research for laboratory facilities placed at the disposal of the first author.

[3] Italic numbers in parentheses refer to Literature Cited, p. 391.

directly with ruthenium red showed the absence of pectin in cell walls of the diseased phloem, parenchyma, and the adjoining cells. Sodium and potassium hydroxide failed to dissolve any part of the necrotic mass, whereas the pectin in healthy cells was dissolved. On treatment with ammonium oxalate, calcium oxalate crystals did not form in the necrotic area, but were abundant in healthy tissue. The zones gave positive tests for pectin.

Lignin.—Place sections in alcoholic phloroglucinol; cover with cover glass and allow a part of the solution to evaporate; add a drop of 25 percent hydrochloric acid at the edge of the cover glass. Lignin stains red violet; soluble in 50 percent chromic acid. Lignin tests on both healthy and diseased cell walls of all tissues were positive.

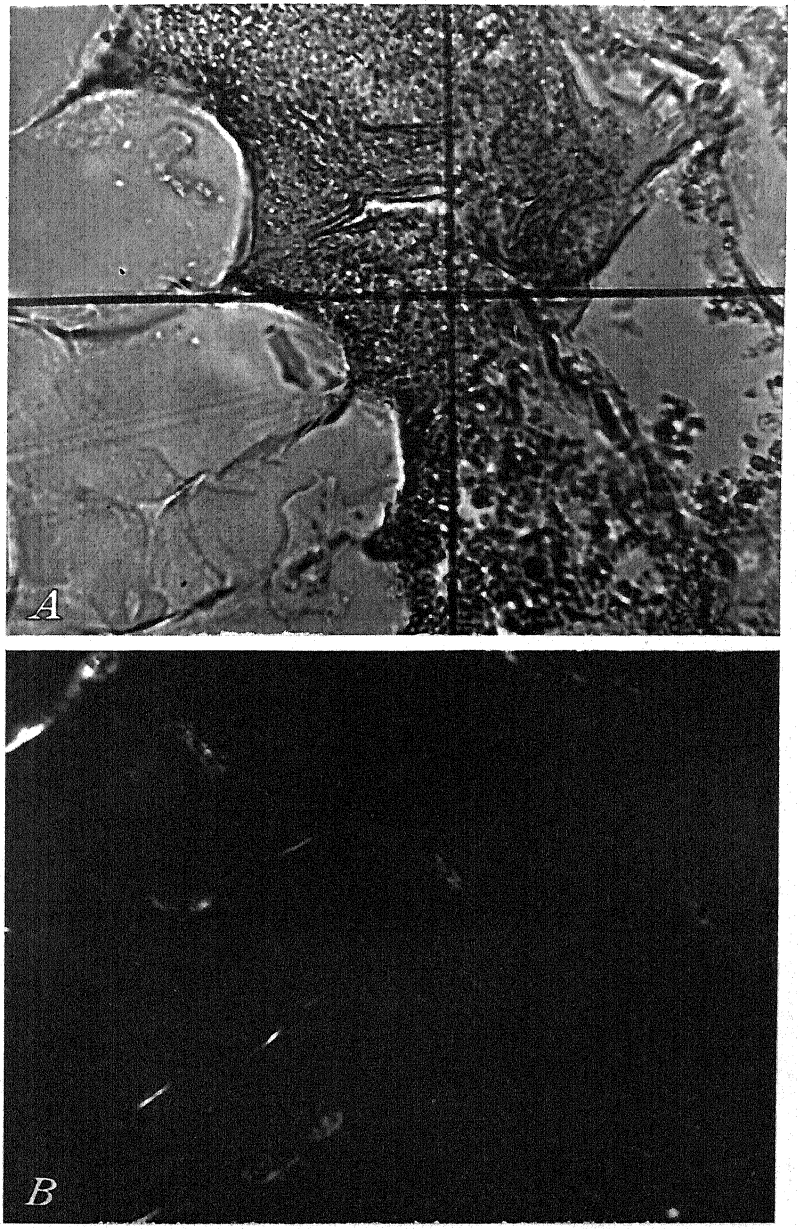
Suberin.—Suberin and suberized deposits are insoluble in 75 percent sulphuric acid, in 50 percent chromic acid, and in zinc chloride and hydrochloric acid; they are soluble in Schultze's reagent, stain red with Sudan III, and gave a sulphur yellow color with potassium hydroxide. Suberin is anisotropic, and the suberized deposit isotropic. The membranes of diseased phloem, parenchyma, and xylem, together with the periderm and cork cells adjoining the rhizome attachment, gave a positive test for suberin. The suberized deposit associated with all necrotic areas, stained with Sudan III and was insoluble in chromic acid, sulphuric acid, and a solution of zinc chloride dissolved in hydrochloric acid. In Schweitzer's reagent the suberized deposit was insoluble and it gave a sulphur-yellow color with potassium hydroxide. Suberin was anisotropic and suberized deposit isotropic (pl. 3, *A* and *B*, and pl. 4, *A* and *B*).

Necrotic areas treated with Schultze's reagent dissolved out the suberized deposit with the formation of fatlike drops which flow together like fatty oils, thus showing the presence of cerin in necrotic regions (pl. 2, *A*). Cerin was also present in the zones, in the periderm, and in suberized cells at stolen attachment. The dissolution of cerin in the periderm left the cellulose wall intact but faintly anisotropic in comparison with the cellulose of the healthy parenchyma. Healthy tissues gave a negative test for cerin.

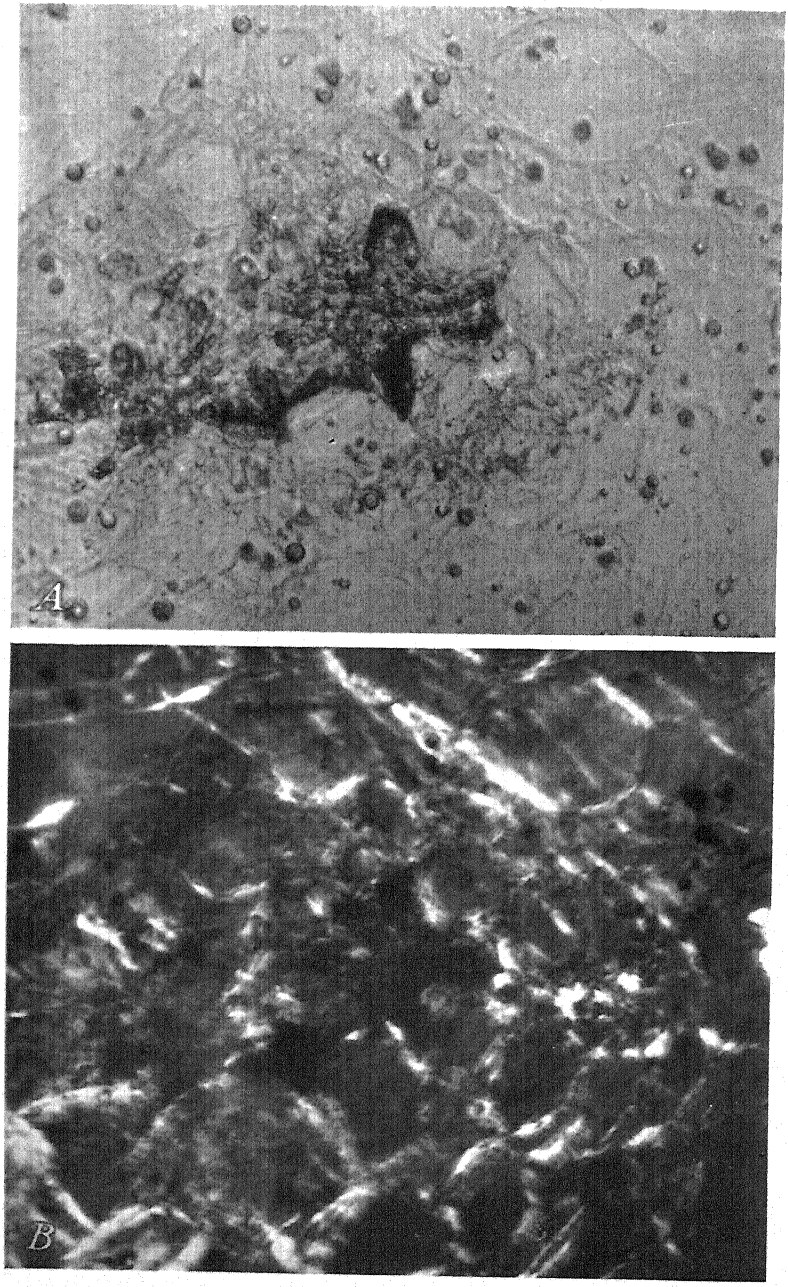
Diseased tubers cut through the necrotic zone and placed in a moist chamber at room temperature for 6 days do not form wound periderm, whereas healthy tubers do, forming 6- to 10-cell layers. This indicates that a periderm is initiated in healthy cells, and that the cells in the necrotic zone have undergone a chemical change as the result of the disease, which prevents periderm formation. There was a normal deposit of suberin on the cut surface of healthy and diseased tubers.

STORAGE SUBSTANCES

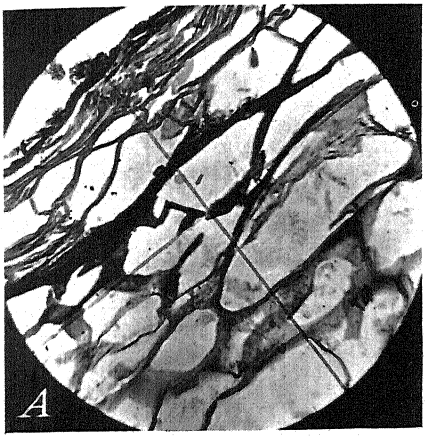
Starch.—Starch grains give a blue color when placed in a weak solution of iodine-potassium iodide. They are birefringent. Orton and Hill (*6*) described an unusual type of starch hydrolysis whereby starch grains undergo a gradual dissolution, become spherical, reduced in size, but retain their characteristic crosses with polarized light until they almost disappear (pl. 3, *C* and *D*). There is a progressive disappearance of the starch from the apparently healthy cells surrounding the zone to the necrotic area which is usually devoid of starch. Starch grains are less numerous in parenchyma cells under the periderm than

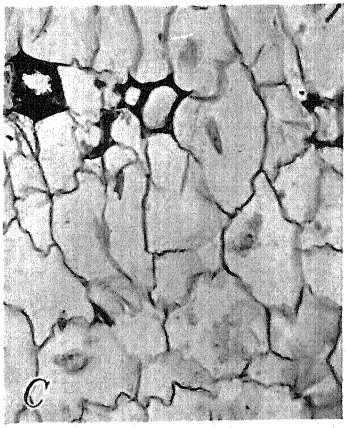


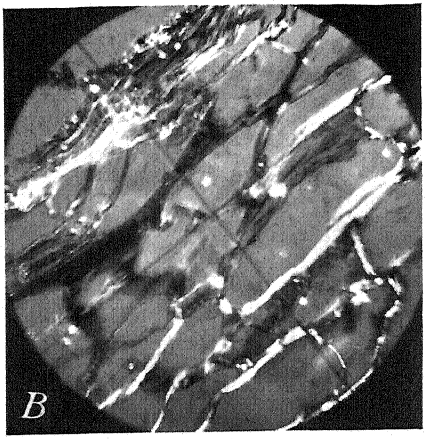
A, Transverse section of necrotic phloem showing suberized deposit. × 780. *B*, *A* with polarized light showing fragments of cellulose walls which were masked by suberized deposit. × 720.

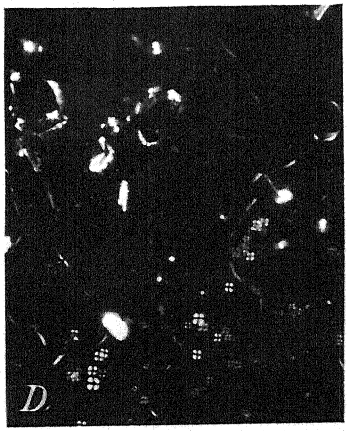


Transverse section of necrotic phloem after 4 hours' treatment in Schultze's reagent: *A*, The oillike drops which are concentrated in the necrotic area gave a positive test for cerin. × 350. *B*, *A* with polarized light showing cellulose remaining intact within necrotic area. × 320.

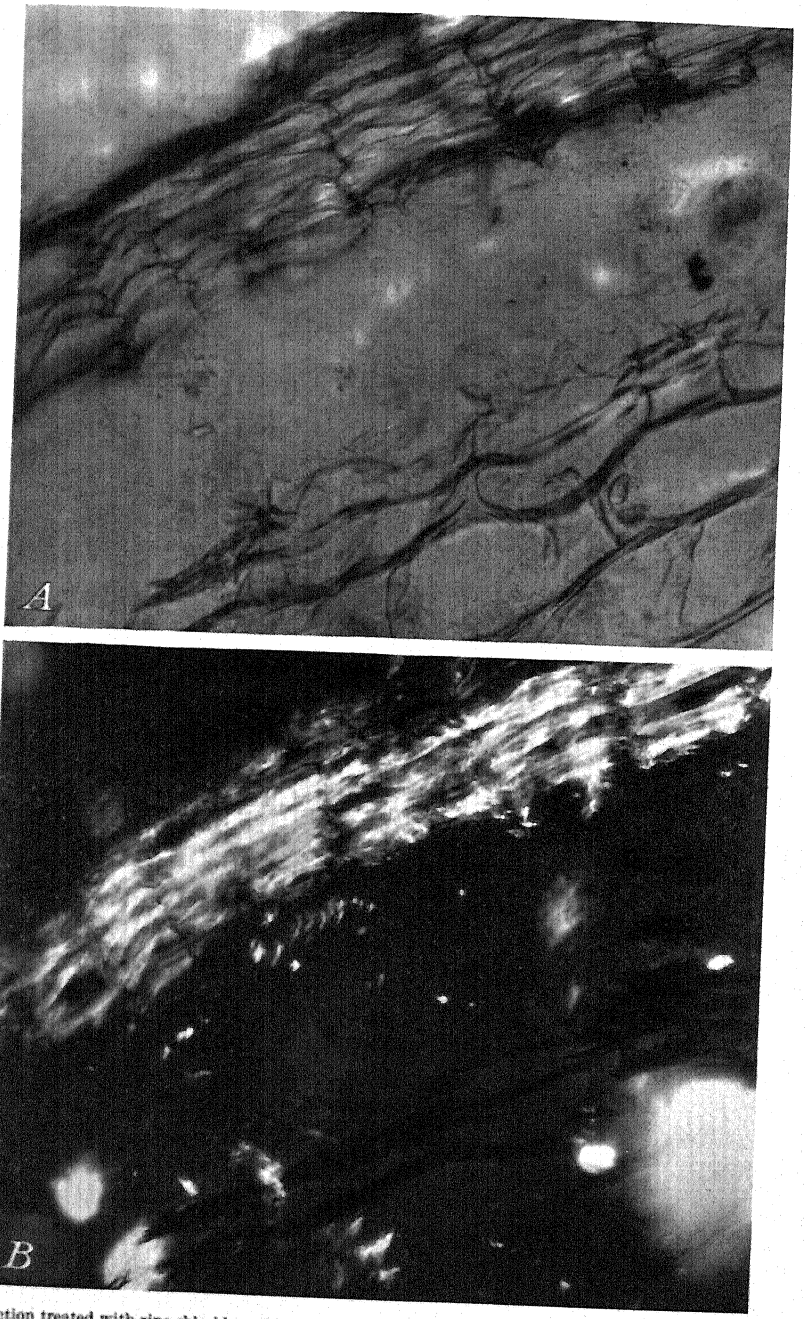




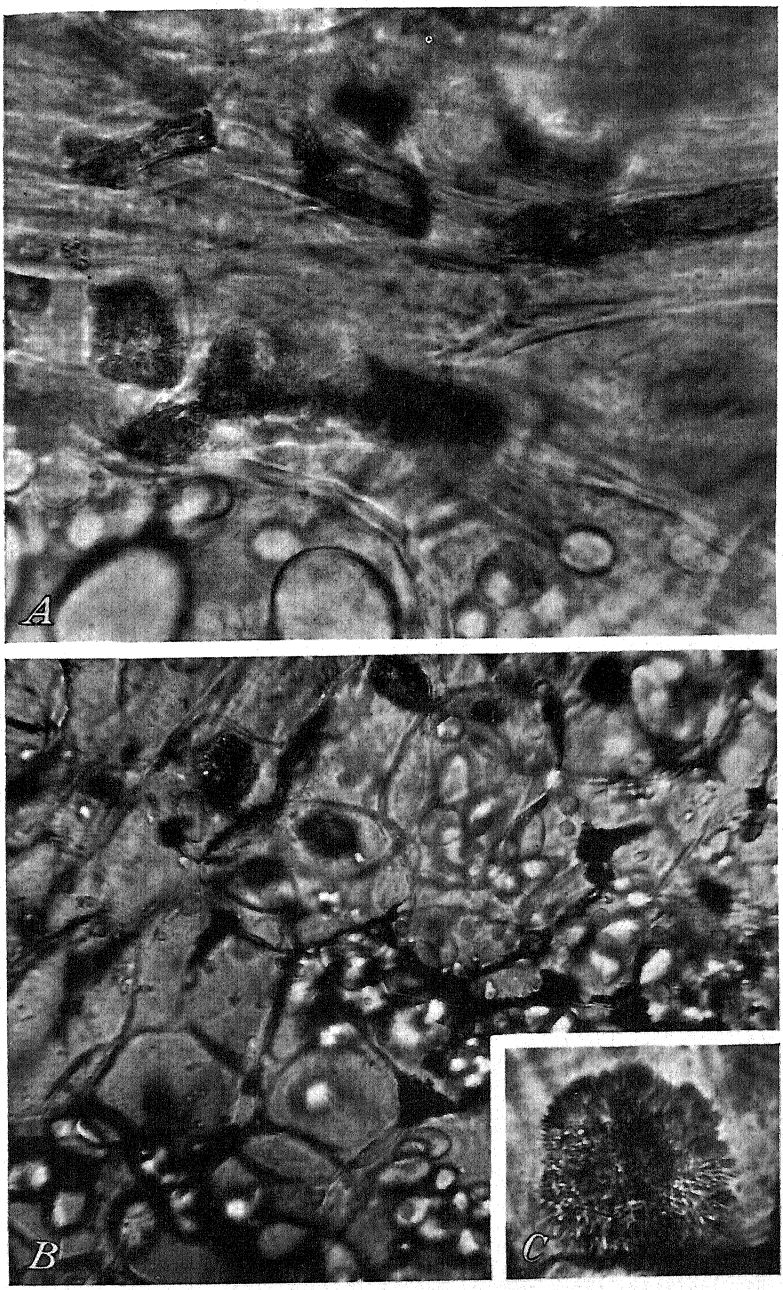




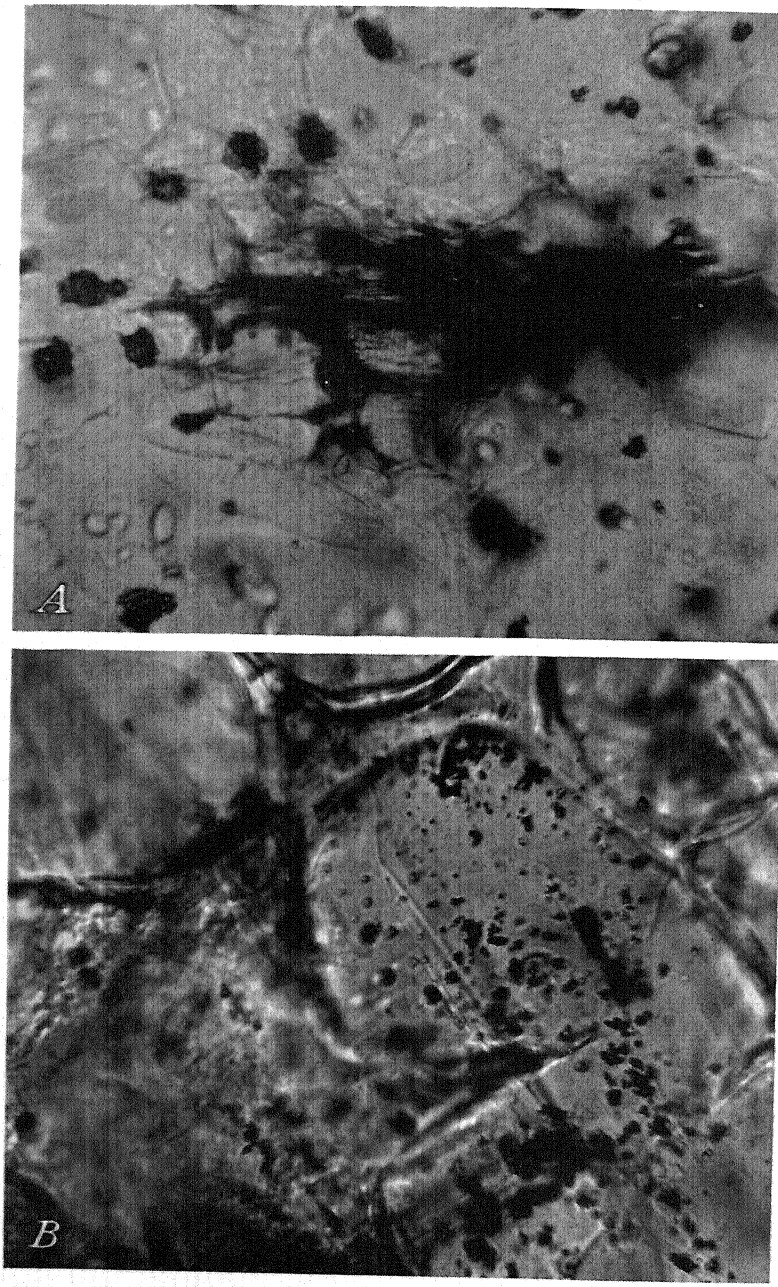
A, Necrotic parenchyma under periderm which gave a positive test for suberin. × 345. *B*, *A* with polarized light showing anisotropic periderm, isotropic suberized deposit, and anisotropic cellulose of the adjoining parenchyma. × 320. *C*, Transverse section of necrotic xylem showing suberized deposit in vessel, and small spherical starch grains aggregated around nuclei. × 360. *D*, *C* with polarized light showing isotropic suberized deposit in vessel, anisotropic cellulose in vessel wall, and small spherical starch grains aggregated around nuclei which have retained their cross until complete dissolution. × 360.



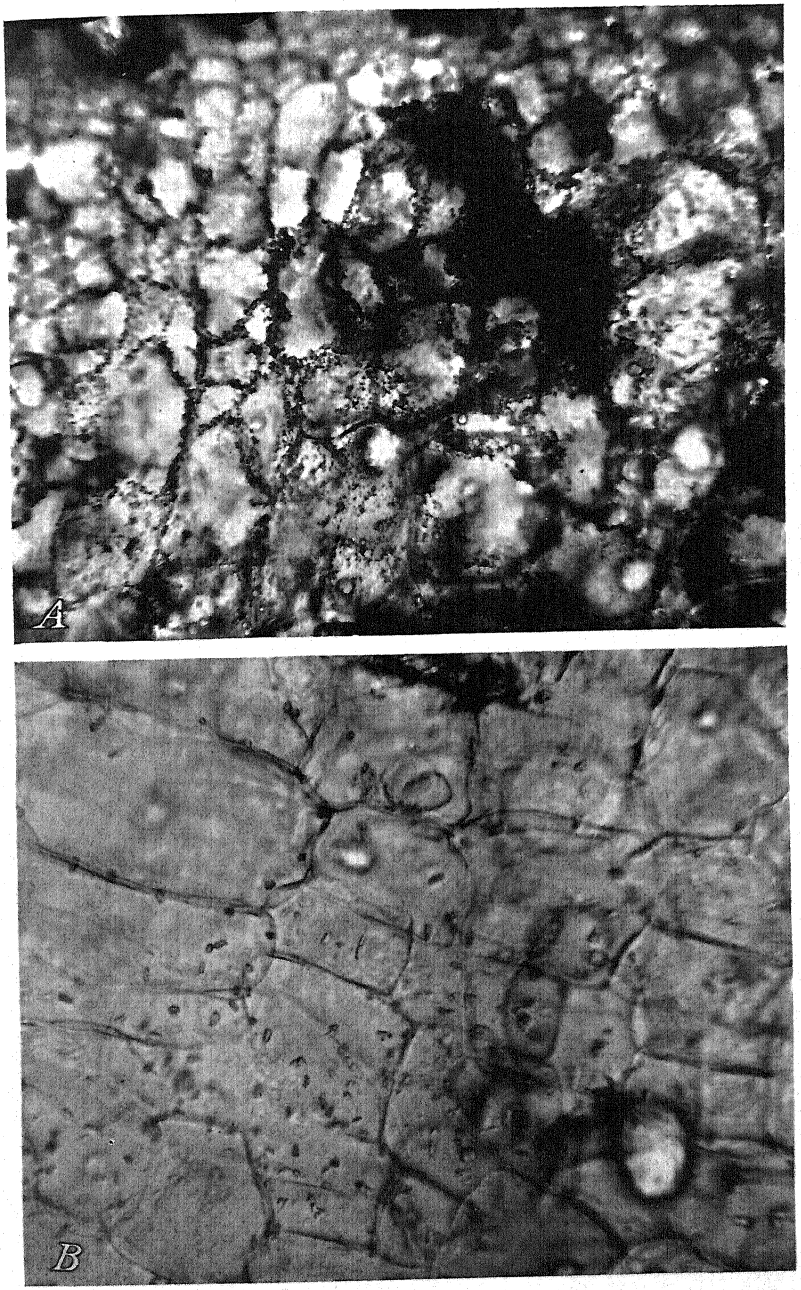
A, Section treated with zinc chloride and hydrochloric acid for 4 hours which dissolved the cellulose under the periderm. Longitudinal section near rhizome attachment showing periderm and suberized layer in parenchyma cells adjoining vascular region. × 350. *B*, *A* with polarized light showing anisotropic periderm and isotropic suberized deposit. The large anisotropic substances are starch grains out of focus. × 320.



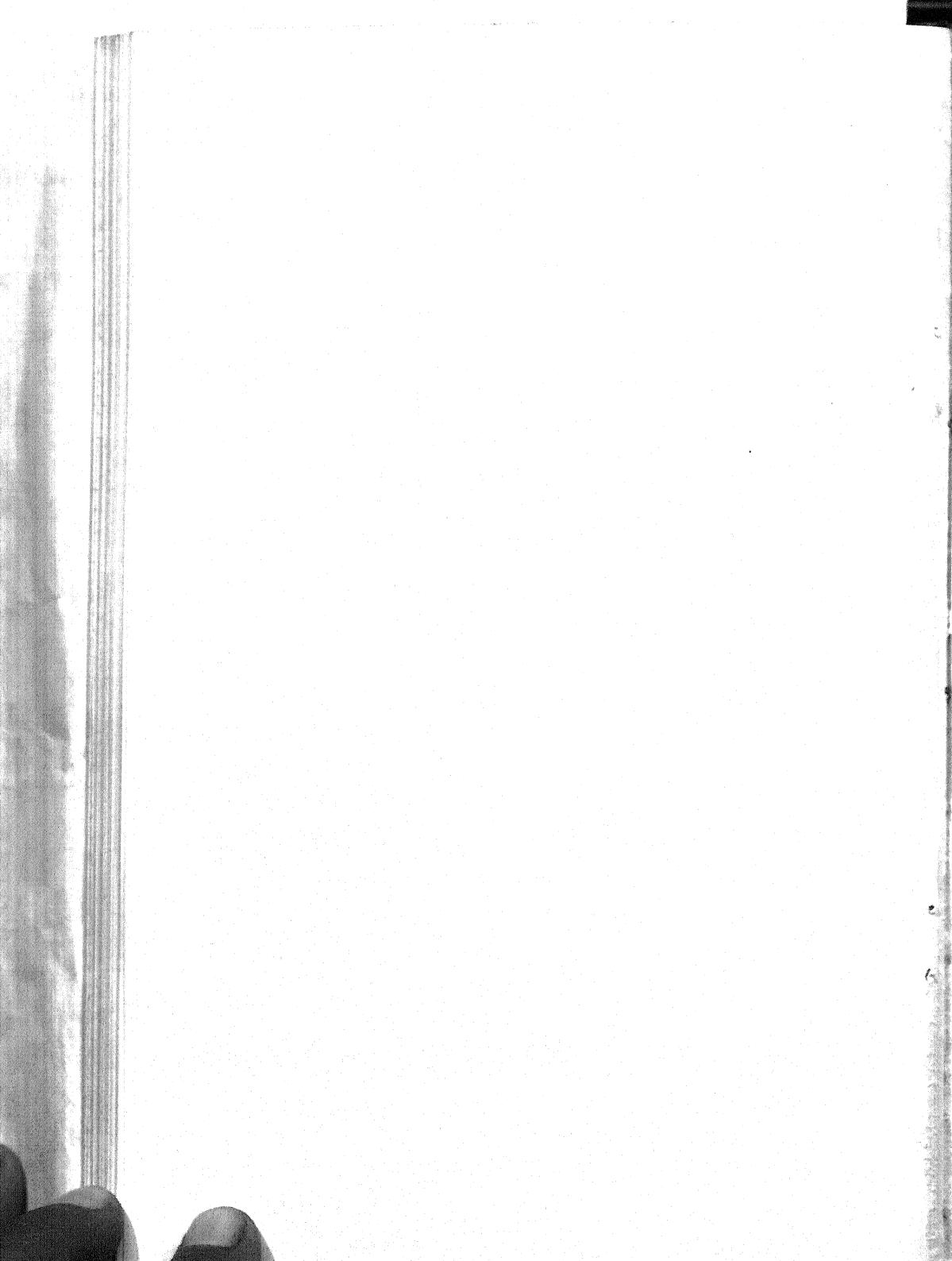
A, Longitudinal section through healthy phloem showing glucosazone formation in sieve tubes. Note the starch grains in parenchyma adjoining phloem. × 415. *B*, Healthy parenchyma tissue showing glucosazones. × 270. *C*, Glucosazone in healthy parenchyma cell. × 415.



A, Transverse section of necrotic phloem showing glucosazones. × 270. B, Cuprous oxide crystals in parenchyma cells adjoining necrotic phloem. × 415.



A, Transverse section through a necrotic region showing potassium cobalt nitrite crystals in the zone. × 195. *B*, Longitudinal section through diseased parenchymatic zone showing ammonium magnesium phosphate crystals. × 330.



in other storage cells and when found in the eye tissues they are usually spherical and resemble the storage starch in the aerial parts of the potato plant.

Glucose.—Warm the sections in Flückiger's reagent for 2 to 4 minutes; glucose indicated by a precipitate of cuprous oxide. Glucosazones are formed when sections are treated with phenylhydrazine hydrochloride, 1 drop, and sodium acetate, 2 drops, for 24 hours. Glucose was present in parenchyma (pl. 5, *B* and *C*), phloem, and xylem tissues of healthy and diseased tubers, and occurred in high concentrations in the zones surrounding necrotic areas (pl. 6, *A* and *B*).

Glucosazones are more abundant in the sieve tubes of the healthy tubers than in other tissues (pl. 5, *A*). This is an indication of the role of the sieve tubes in the translocation of glucose. (Note the crystals at the end of the glucosazones within the sieve tubes; the sieve tube wall has prevented the normal sperical formation of the osazones.)

Sucrose.—Glucose is removed with Flückiger's reaction and washed out of the cells with 5 percent tartaric acid, warmed in concentrated magnesium chloride, and washed in 5 percent tartaric acid. Sucrose is inverted with invertase or weak acid and tested for glucose. Sucrose was found in very small quantities in both healthy and diseased tubers, with a slightly higher concentration within the diseased phloem cells and adjoining zones. It was more abundant within the phloem regions of both healthy and diseased tubers than in the storage tissue.

Fat.—Place sections in Sudan III for 20 minutes and wash with 50 percent alcohol. All fatty substances stain red. When sections are placed in 10 percent potassium hydroxide many small globules appear, showing Brownian movement.

Place sections on a slide in a few drops of saponifying reagent (equal volumes of concentrated potassium hydroxide and 20 percent ammonia) and seal the cover glass with wax. Saponification begins after a few hours and continues for several days. Fat occurs uniformly throughout diseased and healthy tubers. The yellow precipitate which is associated with necrotic regions gave a negative test for fat. Healthy and diseased tissues placed in several changes of acetone for 10 days and tested for fat gave a negative test.

Protein.—After keeping in a 5-percent solution of copper sulphate for 30 minutes, wash the sections with water and place on a slide in a drop of 50-percent potassium hydroxide. Proteins give a red to blue-violet color. In Millon's reagent the protein containing tyrosine becomes vermillion red. Proteins give a yellow precipitate with a weak solution of iodine-potassium iodine. Protein was absent in the necrotic areas and the zones but present in healthy tissues, with greater concentration in the "eyes." Tyrosine was detected with Millon's reagent in healthy tissue, in the apparently healthy cells of diseased tubers, occurring in greater concentration in the eyes; it was absent in the necrotic areas and in the zones.

Solanine.—Cells containing solanine give a red color in a solution of sodium sulphate in sulphuric acid, while in sulphuric acid solanine first gives a raspberry color, then changes to dark violet, and finally becomes colorless. Healthy tissues gave a positive test. Necrotic regions and zones gave a negative test.

MINERALS

Calcium.—Place tissue on a slide; run simultaneously a drop of 5 percent sulphuric acid and a drop of water under the cover glass for the formation of calcium sulphate crystals. Calcium oxalate crystals are detected by treating for 30 minutes with a 2 percent solution of oxalic acid, and by adding a drop of alcohol to the edge of cover glass. Calcium was found in equal quantities in both healthy and diseased tubers. Calcium oxalate (crystal sand) was found uniformly distributed in the tuber, with cubical calcium oxalate crystals more abundant in the parenchyma tissue near the periderm.

Potassium.—Yellow crystals of potassium chloroplatinate are formed in a 10 percent solution of platinum chloride. Place sections in a solution of sodium cobalt nitrite; a yellow crystalline precipitate of potassium cobalt nitrite is formed in the presence of potassium. Both healthy and diseased tubers contained the same amount of potassium (pl. 7, *A*).

Phosphate.—A solution of magnesium sulphate, ammonium chloride, and water yields crystals of ammonium magnesium phosphate, while ammonium phosphomolybdate crystals are formed in ammonium molybdate in nitric acid. Small quantities of phosphates were distributed uniformly in healthy and diseased tubers.

Nitrates.—Treat dry sections with diphenylamine solution (1 percent of diphenylamine in 75 percent sulphuric acid). The presence of nitrates is indicated by a blue color, whereas brucine-sulphuric acid gives a red color for nitrates. Nitrates were absent in necrotic areas and in the zones. In healthy tubers and apparently healthy cells of diseased tubers nitrates were uniformly distributed in storage tissue but were more concentrated in the eyes.

Magnesium.—To a saturated solution of ammonium chloride in water add a few drops of ammonia, and crystals of sodium phosphate to make a 0.1-percent solution. This is warmed and allowed to stand for 10 minutes. Ammonium magnesium phosphate crystals appear when magnesium is present. Magnesium was uniformly distributed in storage tissue of both healthy and diseased tubers. The absence of starch within the necrotic regions made it possible to show the localization of magnesium within the cells (pl. 7, *B*).

Sulphates.—A 10-percent barium chloride solution is the reagent used for sulphates which appear in the form of barium sulphate. Small, glistening, scalelike crystals of benzidene sulphate appear when the cells are treated with benzidene chloride. Sulphates were found in very small quantities in both healthy and diseased tubers.

Chloride.—A 5-percent solution of silver nitrate precipitates silver chloride. Thallium chloride crystals form in a 2-percent thallium sulphate solution. Chlorides were present in minute quantities in both healthy and diseased tubers.

Iron.—After treating the cells with a 2-percent solution of potassium ferrocyanide for 15 minutes a drop of 2-percent hydrochloric acid is added and then washed with water. Iron is indicated by a dark-blue precipitate of ferric ferrocyanide. After treating the cells for 15 minutes in a 2-percent solution of potassium ferricyanide, a drop of 2 percent hydrochloric acid is added and washed with water. Iron is indicated by a dark-blue precipitate of ferrous ferrocyanide. Iron was concentrated in the zones. Ferric and ferrous ferrocyanide were

precipitated in the zones. In healthy tissues a blue color indicated a uniform presence of iron. A precipitate of ferric and ferrous ferrocyanide was associated with the plastids.

PHENOL

Millon's reagent gives a cherry-red color, and Liebermann's reagent produces a red color. Phenol was detected in the necrotic areas of phloem, xylem, and parenchyma; it was absent in the zones and in the apparently healthy cells of diseased tubers as well as in healthy tubers.

OXIDASE

Guaiaconic acid gives a blue color; after 15 minutes in a dilute solution of benzidine, the cells containing oxidases become blue. Oxidase was concentrated in the zones. In both diseased and healthy tubers oxidase was also concentrated in the eye tissues and in the cortex under the periderm. Guaiaconic acid applied directly to a freshly cut diseased tuber gave a blue color in the zones. Benzidine applied to freshly cut tubers produced a purplish-blue color in the zones after standing for 15 minutes.

SUMMARY

Comparative microchemical tests on potato tubers infected with blue stem disease gave the following results: Cellulose and pectic cell walls were partially masked in the necrotic regions of phloem and parenchyma by a deposit of suberin. When the suberized deposit was dissolved with an oxidizing agent the cellulose walls remained intact. Cellulose and lignin gave positive tests in walls of necrotic xylem. A suberinlike substance which was detected in necrotic phloem, parenchyma, and xylem was soluble in Schultze's reagent and gave a positive test for cerin

Starch grains were partially or totally dissolved in the necrotic areas and in the zones and were replaced by a higher concentration of glucose, with no abnormal changes in sucrose content. Protein, tyrosine, and solanine were absent in necrotic areas and in the zones. Fat was found uniformly distributed throughout the healthy tubers as well as in the zone of diseased tubers, but was absent in the necrotic areas.

Calcium, potassium, phosphates, magnesium, chlorides, and sulphates were found in very small quantities in both healthy and diseased tubers. No nitrates were found in the necrotic areas and in the zones. Iron and oxidase were concentrated in the zones. Phenol was detected in the cell walls of the necrotic areas.

LITERATURE CITED

(1) CHAMOT, EMILE MONNIN.
1921. ELEMENTARY CHEMICAL MICROSCOPY. Ed. 2, partly rewritten and enl., 479 pp., illus. New York.

(2) ——— and MASON, CLYDE WALTER.
1930–31. HANDBOOK OF CHEMICAL MICROSCOPY. 2 v., illus. New York and London.

(3) EMICH, FRIEDRICH.
1932. MICROCHEMICAL LABORATORY MANUAL. Transl. by Frank Schneider. 180 pp. New York and London.

(4) HINRICHS, CARL GUSTAV.
1904. FIRST COURSE IN MICROCHEMICAL ANALYSIS. With atlas. 156 pp., illus. St. Louis, New York and Leipzig.
(5) MOLISCH, HANS.
1921. MIKROCHEMIE DEN PFLANZE. Aufl. 2, neubearb., 434 pp., illus. Jena.
(6) ORTON, C. R., and HILL, L. M.
1937. AN UNDESCRIBED POTATO DISEASE IN WEST VIRGINIA. Jour. Agr. Research 55: 153–157, illus.
(7) TUNMANN, O.
1913. PFLANZENMIKROCHEMIE; EIN HILFSBUCH BEIM MIKROCHEMISCHEN STUDIEN PFLANZLICHER OBJEKTE. 631 pp., illus. Berlin.

A COMPARISON OF THE VITAMIN D POTENCY OF THE STEMMY AND LEAFY PORTIONS OF ALFALFA HAY[1]

By G. C. WALLIS

Assistant dairy husbandman, South Dakota Agricultural Experiment Station

INTRODUCTION

Experimental work in several laboratories has shown that various kinds of hay carry measurable amounts of vitamin D. Steenbock and his coworkers (*7*)[2] were the first to use recognized vitamin D assay methods to demonstrate that clover hay had a measurable amount of this factor, and that the potency varied significantly with the curing history. Russell (*5*) and Smith and Briggs (*6*) have shown that these observations also apply to alfalfa hay. Using rats as the test animal, they found little or no antirachitic activity in the leaves from samples of alfalfa hay cured in the dark, but demonstrated an increasing vitamin D potency in the leaves from samples of hay that were exposed to increasing amounts of sunshine during and after the curing process. Wallis, Palmer, and Gullickson (*8*) not only showed by standard line-test technique that prairie hay bought on the open market had a measurable amount of vitamin D, but they also demonstrated that it was of importance in preventing and curing the symptoms of vitamin D deficiency in young calves. Bechdel, Landsburg, and Hill (*1*) have also measured the vitamin D in hay and demonstrated its efficiency in preventing and curing rickets in calves. In a rather extensive study Huffman, Duncan, and Lightfoot (*3*) demonstrated somewhat quantitatively the antirachitic value of sun-cured timothy and alfalfa hay in the ration of dairy calves, while Rupel, Bohstedt, and Hart (*4*) and Gullickson, Palmer, and Boyd (*2*), also using calves, showed that hay carries some vitamin D, and demonstrated its importance in preventing the development of rickets-like symptoms in young growing calves.

During the processes of curing and handling alfalfa hay the leaves often shatter badly from the stems. So far as the writer is aware, no studies have been reported to indicate whether the vitamin D is distributed evenly throughout the plant or is concentrated more heavily in certain portions. The present investigation was therefore designed to study the vitamin D activity of the stems as compared with that of the leafy portion of a sample of alfalfa hay.

EXPERIMENTAL METHODS AND RESULTS

The alfalfa hay used in this experiment was taken from the mow of the dairy barn, and represented a random sampling from the regular herd supply. It was a good quality green-colored sample from a crop

[1] Received for publication March 14, 1938, issued September 1938.

[2] Italic numbers in parentheses refer to Literature Cited, p. 395.

which had been cut on June 15, cocked within a day or two, and hauled to the barn between June 29 and July 3, 1937. It received one light shower of rain. After the sample had been obtained, the leaves were stripped carefully from the stems by hand and were found to represent 49.4 percent of the weight of the hay, leaving 50.6 percent for the stems. Each sample was ground separately to a fine powder in a Wiley mill, placed in sealed glass jars, and stored in a refrigerator until the assay was completed during the next 2 months.

The regular "line-test" technique was used for evaluating the vitamin D content of these samples. The samples were assayed at 1-, 2-, and 3-g levels by taking the required amount of the material and incorporating it in sufficient of Steenbock's rachitogenic diet No. 2965 to make a total of 40 g. This 40-g portion was usually consumed during the first 7 or 8 days, and the rachitogenic diet only was given for the remainder of the 10-day period. Two standard reference groups of rats were assembled during the assay of each material, one group receiving a total of 5 International units of vitamin D and the other, 8 International units. Large litters were distributed evenly over the various levels of feeding for the leaves, stems, and reference oil. Smaller litters were spread evenly over the groups on reference oil and one of the samples being assayed. The results of these assays are summarized in table 1.

TABLE 1.—*The vitamin D potency of the stems and leaves of alfalfa hay as compared with standard reference oil*

Material and quantity	Rats [1]	Average healing [2]	Calculated vitamin D per gram
	Number		*International units*
Standard reference oil:			
5 International units	10	+0.98	
8 International units	9	+1.58	
Leaves from alfalfa hay: 1 g	9	+2.06	10.45
Standard reference oil:			
5 International units	11	+.91	
8 International units	9	+1.64	
Stems from alfalfa hay:			
1 g	10	+.26	
2 g	11	+.64	
3 g	10	+1.03	1.72

[1] The first-mentioned reference-oil groups of rats were litter mates of those receiving the alfalfa leaves; the reference-oil groups mentioned later were litter mates of those receiving the alfalfa stems.
[2] A narrow, continuous line is evaluated as a 1+ healing.

From the results of the assay it is evident that 1 g of the leaves from this sample of alfalfa contained somewhat more than 8 International units of vitamin D. Calculations from the responses obtained indicate an approximate potency of 10.45 International units per gram of leaves. On the other hand, 3 g of the stems gave considerably less response than 8 International units of vitamin D and only slightly more than 5 International units. Two grams of the stems gave appreciably less response than 5 International units. Calculations from these figures indicate an approximate potency of 1.72 International units of vitamin D per gram of stems. On this basis the leaves are almost exactly six times as potent as the stems in the antirachitic factor.

This relationship in potency between the leaves and the stems is also roughly indicated by another line of reasoning from the above data. One gram of leaves gave an average healing of 2.06+ while 3 g of stems fed to rats, many of which were litter mates, gave an average healing of only 1.03+, or exactly one-half of the amount. While the numerical values assigned to the healing responses are not necessarily in proportion to the units of vitamin D fed, still in this experiment the average healing response shown by fairly large groups of rats assembled by assigning litter mates to various levels of vitamin D feeding was approximately proportional to the vitamin D received. This relationship held most closely over ranges of healing where differences in response to graded doses of vitamin D could be most accurately evaluated, as between about 0.5+ and 2.0+ healing. For example, in the reference groups used with the leaves, 5 International units of vitamin D gave an average healing of 0.98+ and 8 units showed a proportionate response of 1.58+. If this observation is applied to the healing data for the rats on the stems and leaves where three times the amount of material gave only one-half the healing response it again gives a rough indication that the leaves are approximately six times as potent as the stems.

If the superior potency of the leaves over the stems which was found in this sample of alfalfa hay proves to be quite generally true as more samples are tested, it provides still another reason why care should be exercised to conserve the leafy portions in the processes of making and handling alfalfa hay. It also indicates that alfalfa leaf meal would have special value in the rations of young growing animals such as calves, pigs, and chickens when the conditions are such that a vitamin D deficiency is likely to occur.

SUMMARY

A representative sample of good quality green-colored alfalfa hay was carefully divided into its leafy and stemmy portions. The leaves represented 49.4 percent and the stems 50.6 percent of this sample of hay. The vitamin D potency of these two portions was obtained by using the standard line-test technique. The leaves were found to be about six times as potent in vitamin D as the stems. They contained approximately 10.45 International units of vitamin D per gram, whereas the stems contained only 1.72 International units per gram.

LITERATURE CITED

(1) Bechdel, S. I., Landsburg, K. G., Hill, O. J., and others.
1933. rickets in calves. Pa. Agr. Expt. Sta. Bull. 291, 41 pp., illus.
(2) Gullickson, T. W., Palmer, L. S., and Boyd, W. L.
1935. a rickets-like disease in young cattle. Minn. Agr. Expt. Sta. Tech. Bull. 105, 47 pp., illus.
(3) Huffman, C. F., and Duncan, C. W., with the technical assistance of Lightfoot, C. C.
1935. vitamin d studies in cattle. i. the antirachitic value of hay in the ration of dairy cattle. Jour. Dairy Sci. 18: 511–526.
(4) Rupel, I. W., Bohstedt, G., and Hart, E. B.
1933. vitamin d in the nutrition of the dairy calf. Wis. Agr. Expt. Sta. Research Bull. 115, 40 pp., illus.
(5) Russell, Walter C.
1929. the effect of the curing process upon the vitamin a and d content of alfalfa. Jour. Biol. Chem. 85: 289–297.

(6) Smith, Margaret Cammack, and Briggs, Ian A.
1933. THE ANTIRACHITIC VALUE OF ALFALFA AS AFFECTED BY EXPOSURE TO SUNSHINE IN THE CURING PROCESS. Jour. Agr. Research 46: 235–240.

(7) Steenbock, H., Hart, E. B., Elvejhem, C. A., and Kletzien, S. W. F.
1925. DIETARY FACTORS INFLUENCING CALCIUM ASSIMILATION. VI. THE ANTIRACHITIC PROPERTIES OF HAYS AS RELATED TO CLIMATIC CONDITIONS WITH SOME OBSERVATIONS ON THE EFFECT OF IRRADIATION WITH ULTRA-VIOLET LIGHT. Jour. Biol. Chem. 66: 425–440, illus.

(8) Wallis, G. Carroll, Palmer, L. S., and Gullickson, T. W.
1935. THE RELATION OF VITAMIN D TO CALCIUM AND PHOSPHORUS RETENTION IN CATTLE AS SHOWN BY BALANCE TRIALS. Jour. Dairy Sci. 18: 213–228, illus.

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 57 WASHINGTON, D. C., SEPTEMBER 15, 1938 No. 6

FOLIAR DIAGNOSIS IN RELATION TO DEVELOPMENT AND FERTILIZER TREATMENT OF THE POTATO[1]

By WALTER THOMAS, *professor of plant nutrition*, and WARREN B. MACK, *professor of vegetable gardening, Pennsylvania Agricultural Experiment Station*

INTRODUCTION

Foliar diagnosis is defined (*1*, *6*) [2] as the chemical condition or state (chemism) of a leaf with respect to the dominant nutritive elements (entities) at the instant of sampling, and taken from a predetermined and suitable position. The annual foliar diagnosis of a plant consists, then, of a sequence of conditions or states with respect to the quantities of the dominant nutritive elements in the leaf, taken from a predetermined position, as revealed by analysis at different stages during the growth cycle from one year to another on the same soil in a given location.

It has been pointed out (*6*) that the keystone of the method of foliar diagnosis rests upon facts experimentally established [3] (*2*, *5*, *7*), which, briefly summarized, are as follows:

Plants growing on two experimental plots which are similarly nourished (as deduced from similar development and yields) are represented in general and with a sufficient approximation by similar foliar diagnoses; and, conversely, plants growing on two plots which are differently nourished (as deduced from different development and different yields) are represented by different foliar diagnoses.

The purpose of the work reported in this paper was to determine relation of the foliar diagnosis of potato plants grown on the vegetable fertility plots of the Pennsylvania Agricultural Experiment Station to the development of plants from duplicate (similarly fertilized) plots and to the fertilizer applied.

The point of departure of the method of foliar diagnosis from the methods of traditional agronomy is that it utilizes as an analytical expression of plant performance not the soil nor the fertilizer applied, but the chemical condition of the leaf at different stages in its life cycle.

MATERIALS AND METHODS

EXPERIMENTAL PLOTS

The potato plants used in the present investigation were grown on tiers 1, 2, 4, and 5 of the station vegetable-fertility plots which were laid out in 1916. A description of the experimental plan has been given by Mack (*3*). The plots [4] are 12 by 36.3 feet and are separated

[1] Received for publication January 18, 1938; issued September 1938. Paper No. 819 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[2] Italic numbers in parentheses refer to Literature Cited, p. 414.

[3] THOMAS, WALTER. COMPOSITION OF LEAVES OF THE SAME PHYSIOLOGICAL AGE, AS DETERMINED PERIODICALLY, OF APPLE TREES GROWN IN METAL CYLINDERS AND SUBJECTED TO DIFFERENT TREATMENTS WITH NUTRIENT SALTS. Unpublished.

[4] To conserve space, where tier and plot are referred to together, two figures are used, the first indicating tier, the second, plot; as, 1-2—tier 1, plot 2.

by guard strips 6 feet wide; the fertilizer treatment of each plot extends to the middle of the guard strip. The area of the plots exclusive of guard strips is one-hundredth of an acre. The experimental crops consist of a 4-year rotation of cabbage, potatoes, tomatoes, and sweet corn. Rye and vetch are used as a cover crop. The fertilizers are applied in different combinations before the last harrowing and the manure (partly rotted horse manure) before the land is plowed. The variety of potato (*Solanum tuberosum* L.) is the Rural Russet. The tiers and plots examined in 1935 together with their fertilizer treatments are given in table 1.

TABLE 1.—*Tiers and plots examined and fertilizer treatment given, 1935*

Tier No.	Plot No.	Fertilizers applied [1]	Amount applied to plot	N, P_2O_5, and K_2O equivalents (pounds)	Symbol and ratio
			Pounds		
1	2	Sodium nitrate	4.0	0.6	N.
4	16				
1	3	Superphosphate	6.25	1.0	P.
4	15				
1	4	Potassium chloride	1.666	.8	K.
4	14				
1	6	Sodium nitrate	4.0	.6	NP.
4	12	Superphosphate	6.25	1.0	
1	7	Sodium nitrate	4.0	.6	NK.
4	11	Potassium chloride	1.666	.8	
1	8	Superphosphate	6.25	1.0	PK.
4	10	Potassium chloride	1.666	.8	
1	10	Sodium nitrate	4.0	.6	NPK, 6:10:8.
4	8	Superphosphate	6.25	1.0	
		Potassium chloride	1.666	.8	
1	15	Rotted horse manure	[2] 600	4.62 (N)	15:10:12.3.[3]
4	3			3.08 (P_2O_5)	
				3.78 (K_2O)	
2	4	Sodium nitrate	6.0	.9	(1.5N) PK, 9:10:8.
5	4	Superphosphate	6.25	1.0	
		Potassium chloride	1.666	.8	
2	8	Sodium nitrate	4.0	.6	N(1.5P)K, 6:15:8.
5	8	Superphosphate	9.375	1.5	
		Potassium chloride	1.666	.8	
2	12	Sodium nitrate	4.0	.6	NP(1.5K), 6:10:12.
5	6	Superphosphate	6.25	1.6	
		Potassium chloride	2.499	1.2	
2	14	Sodium nitrate	2.0	.3	0.5(NPK), 3:5:4.
5	4	Superphosphate	3.125	.5	
		Potassium chloride	.833	.4	
2	10	Sodium nitrate	8.0	1.2	2(NPK), 12:20:16.
5	2	Superphosphate	13.5	2.0	
		Potassium chloride	3.332	1.6	

[1] Fertilizers within braces were all applied to both plots given in previous column.
[2] Containing 65.7 percent of moisture.
[3] Ratio is on a basis of percentages in 1,000 pounds per acre of complete fertilizer.

The complete fertilizer (NPK) has a ratio $N:P_2O_5:K_2O=6:10:8$. Applications (per plot, one hundredth acre) to this plot were at the rate of 4 pounds of $NaNO_3$, 6.25 pounds of superphosphate, and 1.666 pounds of KCl, and this treatment is hereafter referred to as the standard (S) application (*3*).

METHODS OF SAMPLING

Details of the method of sampling have been given in an earlier publication (*6*).

Leaves from stems of plants that were abnormal in relation to the average of the particular experimental plot were not sampled. The first samples were taken as soon as the plants were sufficiently developed to permit the ensemble of leaves in a row to supply the amount

of material sufficient for analysis. The other samples were collected at intervals.

The leaves were dried in a drying oven immediately after being brought from the field and were then ground in a Wiley mill.

PRESENTATION AND DISCUSSION OF RESULTS

It will simplify the interpretation of results to divide the plots according to treatments, as follows:

(1) Plots receiving the standard fertilizer (S) and those with one or two of the elements omitted, viz, plots receiving N, P, K, NP, NK, PK, NPK.

(2) Plots receiving different proportions of N, P, and K in complete fertilizers, i. e., plots receiving complete fertilizers differently equilibrated—viz, plots 0.5 (NPK), 2(NPK), (1.5N)PK, N(1.5P)K, NP(1.5K), and also 30 tons of manure.

APPEARANCE OF THE LEAVES

The appearance of the leaves of plants receiving different treatments was markedly different.

The N plants, which were small and erect, had small leaves, rugose or wavy along the margins, and dark green; many were folded upward along the midrib.

The P plants were a little larger and less erect than the N plants; the leaves were larger and a little lighter in color, but otherwise not very different from those on the N plots.

The K plants were about the same size as those on the N plots, but the leaves were distinctly larger, smoother, and lighter in color.

The NP plants were somewhat larger and darker green that those on the P plots, but similar in other respects.

The NK plants were about the same size as those on the P plots, but the leaves were smoother; they were a little lighter in color than those on the NP plots.

The PK plants were distinctly larger and more spreading than any of the foregoing, and the leaves were fairly large, nearly smooth, and lighter green than those on the other plots, except possibly on the manure plots.

The NPK plants were a little smaller and less spreading than those on the PK plots; the leaves were a little smoother and lighter in color and also little larger than those on the NP plots.

The "manure" plants were the largest of all, and were less spreading and slightly darker green than those on the PK plots; the leaves were folded somewhat, but were little waved along the margins.

The (1.5N) PK plants were about the same in most respects as those on the NPK plots, though they appeared a little less vigorous and slightly darker green.

The N (1.5P) K plants were larger than those on the NPK plots, and the leaves were larger and less folded; the color of the leaves was about the same as that on the NPK plots.

The NP (1.5K) plants were more spreading than those the NPK plots, and the leaves were smoother, larger, and lighter green.

The 0.5 (NPK) plants closely resembled the N plants, but they were a little lighter in color.

The 2 (NPK) plants resembled those on the NP (1.5K) plots, but they were a little larger and darker green, and the leaves were more folded.

DEVELOPMENT OF THE PLANTS

Method of Determining Development

The development of the plants was determined in the following manner:

Plants were taken from the two halves of guard strips adjacent to a given plot, which received the same fertilizer treatment as the plots being sampled. At each date of sampling for plant development, which was within a day or two after the date of leaf sampling for foliar diagnosis, a plant was dug from every third row lengthwise of the plot, and intervening rows were sampled at later dates. Eight plants, half from each guard strip, constituted a sample on each date.

There is a possibility that some of the roots of these plants extended beyond the area receiving the particular fertilizer treatment into the area receiving the treatment of the adjacent plot. If this occurred, the effect was not sufficient to appear in the tops of the plants, which were apparently like those of the plot which they were considered to represent. This method was followed to avoid disturbing plants on the plots proper, the yields of which would very likely have been influenced by the removal of plants, even if corrections had been made for the missing plants.

The roots of the plants were brushed free from adhering soil, and the individual plants were weighed immediately, first entirely and then with tops removed. The data for all underground portions, including roots and tubers, were recorded together. At the first sampling, roots constituted most of the combined weight, but at later samplings the tubers accounted for an increasingly greater part of the weight. Original seed pieces which in many cases were attached to the plants were removed before weighing was done.

The plants developed in a regularly progressive manner without any injurious factors affecting growth. The development of the aerial parts of the plants from duplicate plots varied greatly in some plots, and especially in the following pairs: NPK (Nos. 4–8 and 1–10); PK (Nos. 1–8 and 4–10); K (Nos. 1–4 and 4–14); and (1.5N) PK (Nos. 2–4 and 5–14). The development was more nearly alike in plants from duplicate plots that did not receive potash; that is, in N (Nos. 1–2 and 4–16), NP (Nos. 1–6 and 4–12), and P (Nos. 1–3 and 4–15). This fact suggests that potash nutrition is a dominant factor in determining the great differences in the development of the plants in some of the duplicate plots; it may be related to the differences in the supply of water (*4*) or to other factors associated with soil heterogeneity.

Relation of Rate of Growth During the Early Part of the Growth Period to Final Yield of Tubers

Table 2 gives the weights of the aerial parts and of the tubers on four different dates—July 4, July 25, August 10, and August 24. Table 3 gives the aerial development as an indication of the growth rate during the period from July 4 to July 25, together with the final yield of tubers.

The parallelism within the plots of a tier is within a sufficient approximation for a field experiment. This correlation may not always be found, although it has long been known from field observations that fertilizers have a marked influence on the rate of growth of plants during the early part of their vegetative development. Barring in-

jurious factors later in the season, the general appearance of crops during the early stages has been recognized by agronomists as affording a good basis for the prediction of yields. The impulse received at the beginning of vegetative growth is characteristic of the mineral nutrition during the whole course of development and the yield often confirms this fact. Hence, for comparative studies, more confidence may be placed in the data for the rapidity of growth as an indication of the response to treatment than in the final yields themselves.

TABLE 2.—*Development of tops and of tubers, from potato plants receiving the standard and the incomplete fertilizers*

[Fresh weights per plant]

Treatment	Tier No.	Plot No.	Portion	July 4	July 25	Aug. 10	Aug. 24
				Grams	*Grams*	*Grams*	*Grams*
N	1	2	Aerial	59.9	162.5	311.3	311.3
			Tubers	17.2	61.3	173.7	273.7
	4	16	Aerial	42.0	151.3	232.5	301.3
			Tubers	14.6	41.2	167.5	275.0
NPK	1	10	Aerial	111.1	425.0	402.5	357.5
			Tubers	20.9	175.0	272.5	466.3
	4	8	Aerial	140.8	500.0	517.5	532.5
			Tubers	25.3	170.0	330.0	565.0
PK	1	8	Aerial	108.0	360.0	327.1	320.0
			Tubers	25.1	212.5	310.0	486.3
	4	10	Aerial	89.0	225.7	407.1	425.0
			Tubers	17.4	122.9	331.5	547.5
NK	1	7	Aerial	85.5	325.0	451.3	386.3
			Tubers	23.4	97.5	275.0	481.2
	4	11	Aerial	53.5	355.0	501.3	380.0
			Tubers	12.4	117.5	278.7	336.3
NP	1	6	Aerial	93.8	306.3	273.8	386.3
			Tubers	21.7	112.5	216.2	457.5
	4	12	Aerial	78.4	307.1	240.0	388.8
			Tubers	18.0	92.9	160.0	352.5
K	1	4	Aerial	60.8	230.0	281.3	356.3
			Tubers	17.3	74.3	213.7	481.2
	4	14	Aerial	31.1	94.3	165.7	170.0
			Tubers	13.0	65.7	140.0	201.3
P	1	3	Aerial	75.5	196.3	246.3	200.0
			Tubers	18.5	86.2	210.0	302.5
	4	15	Aerial	53.8	157.5	153.8	125.0
			Tubers	17.8	108.8	168.7	227.5

TABLE 3.—*Relation between rate of growth and final yields from plants receiving the standard and the incomplete fertilizers*

Treatment	Tier No.	Plot No.	Weight addition to aerial portions, per plant, between July 4 and July 25	Class order	Final yield per plot	Class order
			Grams		*Pounds*	
N	1	2	112.6	7	109	7
	4	16	109.3	5	88	5
NPK	1	10	313.9	1	162	2
	4	8	359.2	1	195	2
PK	1	8	252.0	2	148	4
	4	10	136.7	4	208	1
NK	1	7	239.5	3	163	1
	4	11	301.5	2	174	3
NP	1	6	212.5	4	124	5
	4	12	228.7	3	140	4
K	1	4	169.2	5	155	3
	4	14	63.2	7	70	7
P	1	3	120.8	6	114	6
	4	15	103.7	6	88	6

In this case, the coefficient of correlation between the final yield and the growth of the aerial parts between July 4 and July 25, computed by the usual method, is $+0.785 \pm 0.0692$, which may be considered highly significant.

The presentation of another magnitude—in this case growth rate—in addition to that represented by the final yields, is, from an agronomical standpoint, logically superior to the practice of blindly increasing the number of observations of yields from which to calculate the probable error.

COMPOSITION OF LEAVES FROM PLANTS RECEIVING THE COMPLETE STANDARD FERTILIZER AND THOSE WITH EITHER ONE OR TWO OF THE ELEMENTS OMITTED

The results of the analyses for N, P_2O_5, and K_2O on four sampling dates, expressed as percentage of the dry weight of material, are given in table 4 and are represented graphically in figure 1.

TABLE 4.—*Nitrogen, phosphoric acid, and potash content on different dates of the fourth leaf of potato plants receiving the standard and the incomplete fertilizers; expressed on a dry-weight basis*

Treatment	Tier No.	Plot No.	Mineral nutrient	July 7	July 29	Aug. 9	Aug. 24
				Percent	*Percent*	*Percent*	*Percent*
N	1	2	N	5.12	4.42	4.15	3.64
			P_2O_5	.404	.534	.472	.344
			K_2O	3.97	2.54	2.01	1.77
	4	16	N	4.71	4.11	3.75	3.40
			P_2O_5	.424	.502	.458	.362
			K_2O	4.438	3.139	2.635	2.151
NPK	1	10	N	4.98	3.88	3.62	3.14
			P_2O_5	.584	.509	.482	.392
			K_2O	6.589	4.337	4.697	4.348
	4	8	N	4.92	4.36	3.62	3.23
			P_2O_5	.604	.542	.460	.398
			K_2O	6.907	5.129	5.000	4.961
PK	1	8	N	4.55	3.36	3.08	2.82
			P_2O_5	.624	.510	.496	.430
			K_2O	6.759	5.321	5.348	5.038
	4	10	N	4.45	3.64	3.10	2.75
			P_2O_5	.636	.580	.484	.370
			K_2O	6.651	5.193	5.062	5.300
NK	1	7	N	5.10	4.03	3.78	3.26
			P_2O_5	.464	.510	.452	.352
			K_2O	6.628	5.554	5.279	5.457
	4	11	N	5.04	4.31	3.78	3.36
			P_2O_5	.412	.498	.420	.322
			K_2O	6.790	5.968	6.269	6.434
NP	1	6	N	5.14	3.87	3.71	3.36
			P_2O_5	.590	.532	.486	.396
			K_2O	2.531	1.519	1.186	1.085
	4	12	N	5.08	4.20	3.78	3.37
			P_2O_5	.558	.526	.480	.412
			K_2O	4.632	2.635	2.170	1.755
K	1	4	N	4.61	3.87	3.39	2.78
			P_2O_5	.424	.540	.464	.370
			K_2O	6.318	5.217	4.915	5.000
	4	14	N	4.10	3.50	3.20	2.56
			P_2O_5	.432	.452	.432	.328
			K_2O	6.317	5.232	5.496	5.242
P	1	3	N	4.57	3.46	3.24	2.79
			P_2O_5	.592	.521	.520	.450
			K_2O	2.41	1.64	1.29	1.11
	4	15	N	4.30	3.38	3.06	2.34
			P_2O_5	.548	.512	.514	.444
			K_2O	5.15	3.488	3.04	2.35

GENERAL CHARACTERISTICS OF THE GRAPHS OF PLOTS RECEIVING THE COMPLETE STANDARD FERTILIZER AND THOSE WITH EITHER ONE OR TWO OF THE ELEMENTS OMITTED

The differences between the foliar diagnosis of plants from plots receiving different treatments are much greater than between duplicate pairs receiving the same treatment (fig. 1). On soils much more

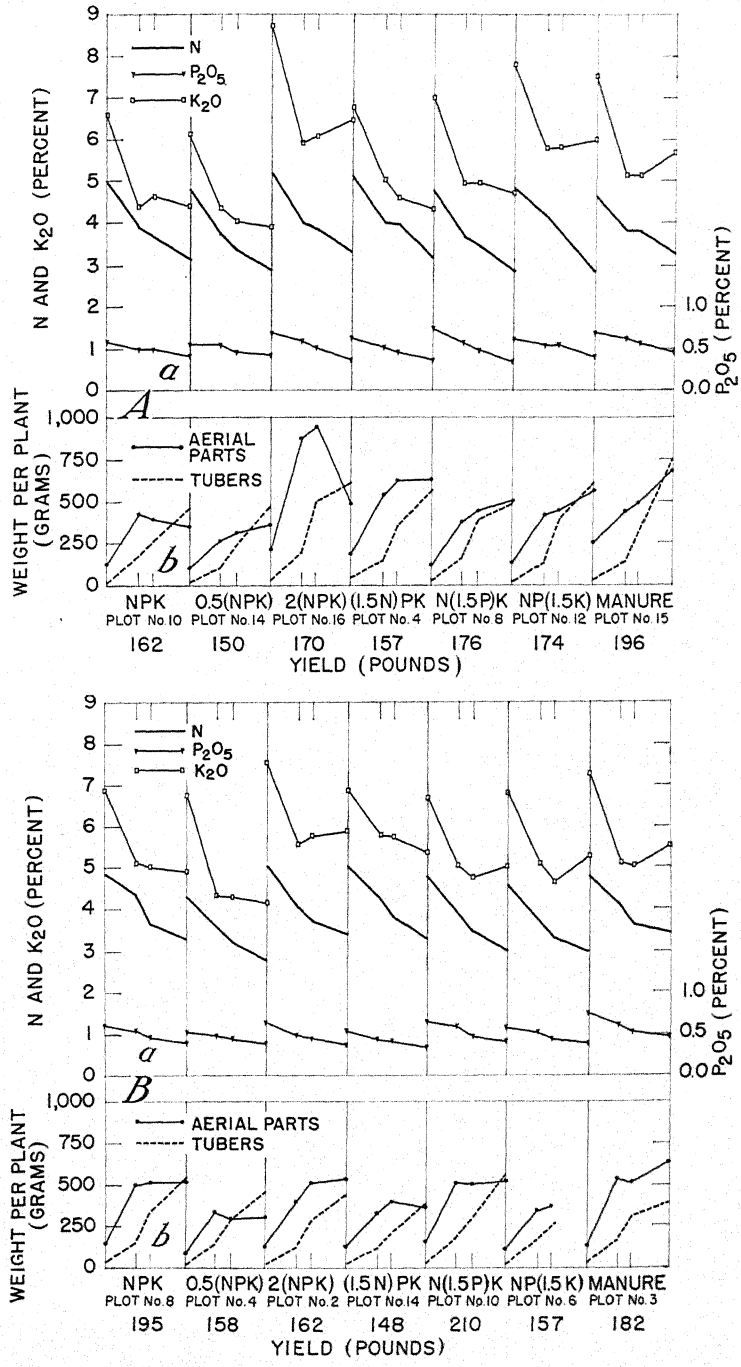


FIGURE 1.—Nitrogen, phosphoric acid, and potash contents of the fourth leaf (dry foliage) of potato plants at four periods of sampling (*a*), and the weights of aerial parts and of tubers at corresponding dates (*b*) from plots receiving the

uniform than the soil of these experiments, it has, as already stated, been established that the foliar diagnoses of plants growing on plots receiving the same treatment, the yields of which are as nearly identical as can be expected under field conditions, coincide.

In the present experiments the foliar diagnoses for nitrogen and phosphoric acid of plants on similarly treated plots are, in general, concordant, within a sufficient approximation for field experiments (as compared with the differences existing between the foliar diagnosis of plants receiving different treatments). The divergences of the graphs for potash in many duplicate plots, however, are very great. This is the result of differences in topography, the exposure of the subsurface clay horizons being greater in some plots than in others. This was accompanied by great differences in the potash nutrition of the plants between certain duplicate plots, especially in the NPK, NP, NK, K, and P plots.

A preliminary examination of the results shows the following characteristics:

Nitrogen.—In all plots of tiers 1 receiving nitrogen addition, the content in terms of the dry weight of the leaf is 5.00 percent (4.71 in tier 4) or more on July 7, the first sampling date, and in all plots which did not receive nitrogen fertilizer applications it is 4.61 (4.45 in tier 4) or less. An increase in the content of N is reflected in all successive samplings in the leaves of plants which received nitrogen additions over those which did not. The nitrogen decreases with the age of the leaf in all cases.

Phosphoric acid.—The phosphoric acid content of leaves from plots in tier 1 treated with superphosphate is 0.584 percent (0.548 in tier 4) or more on July 7, as compared with 0.464 percent (0.424 in tier 4) or less on plots not receiving this fertilizer.

The characteristic of the phosphoric acid graphs in all tiers is the attainment of a maximum on July 29 (second sampling date) on all plots which did not receive phosphate applications. This maximum is not present in the leaves of any plot which received phosphate additions; it appears to be a temperature effect and may be masked in plots which received phosphate additions because of more rapid assimilation. The nitrogen graphs confirm this view, for whenever there is a rapid assimilation of nitrogen, as in the plots which received phosphate, a maximum in the phosphoric acid graphs is not present after the first sampling date.

Potash.—The leaves of plants from all plots which received potash additions contain 6.318 percent (6.317 in tier 4) K_2O or more on July 7, as compared with 3.97 percent (5.15 in tier 4) or less in plots which received no potash fertilizer. These relationships are maintained throughout successive samplings. The potash decreases regularly with the age of the leaf except that when insufficient nitrogen or phosphoric acid is present the latter accumulates in the leaf during the later stages of growth.

The effect factors of each element (N or P or K) are related to the presence of the others in such a way that:

(1) The omission of nitrogen from the complete fertilizer (S) has resulted (compare NPK and PK) in an increase in both the potassium and phosphorus content of the leaf in tier 1 throughout the whole period, and in tier 4 in an increase of the phosphorus through the first three periods, and of the potassium in the last three periods.

The omission of nitrogen from the complete fertilizer is accompanied by a retardation in the development of the aerial parts in both tiers 1 and 4.

(2) The omission of phosphorus from the standard complete fertilizer (S) is accompanied (compare NPK and NK) by an increase in the nitrogen and potassium content, but, as the slopes of the graphs indicate, with smaller utilization of both elements.

(3) The omission of potassium from the complete fertilizer (S) is accompanied (compare NPK with NP) by a slight increase in the nitrogen and phosphorus content of the leaf throughout the period. Associated with this is a generally slower development of the aerial parts and decrease in yeild of tubers. The omission of potassium has resulted in a much lower content of potassium in plot 1–6 than in its duplicate, 4–12, resulting in much lower yields.

Reasons for Comparing the Foliar Diagnosis of Plants on Each Plot With That of Plants on the N Plot

When the experimental plots were laid out in 1916 each tier contained five unfertilized check plots, viz, plot 1 and each fourth plot following it. In 1923, however, and in succeeding years until 1932, the check plots were fertilized uniformly with 2.67 pounds of sodium nitrate and 6.25 pounds of 16 percent superphosphate. A comparison of the yields for the two periods 1918–20 and 1921–22 (*3*) shows that the N plots, which were superior at first (1918–20) to the check plots, later became inferior to them (1921–22). The foliar diagnosis of the plants on the N plots supplies the explanation. The progressive addition of nitrogen has thrown the relationships of N, P_2O_5, and K_2O out of balance, by reducing the absorption of P and K.

In the following analysis of data of tier 1, therefore, all of the plots from No. 3 to No. 10 will be compared with plot No. 2 of this tier, viz, the N plot, since this is the lowest yielding plot. Before doing so, the N plot (1–2) will be discussed in detail to illustrate the characteristic properties of the graphs (foliar diagnosis). It will be shown that the differences in foliar diagnosis between the differentially treated plots correspond to important differences in the mode of vegetative growth and of the development of tubers.

Foliar Diagnosis of Plants on the N Plot as Illustrative of the Method of Interpretation

The graphs for the N plot represent the foliar diagnosis, as defined (p. 397), for N, P_2O_5, and K_2O of the Rural Russet variety for the year 1935. The foliar diagnosis represents an analytical characteristic of the chemism or chemical state of the plants on the N plot (No. 2) of tier 1 at the moment of sampling the leaves. This chemical condition is based on the dry matter of the leaf without taking into consideration the weight of the dry material at each sampling or the number of leaves sampled from each plant.

It is now clear from the definitions that no physiological significance whatever can be attributed to the graphs of the N plot when considered alone, but only in its relation to other plots. Hence, the graphs considered independently of all other field data and all other foliar diagnoses have no physiological or agronomical significance.

Certain inferences, however, may be drawn from the graphs of the N plot (1–2). For example, the foliar diagnosis of the plants on this

plot shows the lowest phosphoric acid content and the poorest assimilation of this element of any of the plots, and is accompanied by slow development of the plants; small, wrinkled, dark-green leaves; and low yield. Furthermore, these characteristics are reproduced in the duplicate N plot (4–16).

COMPARISON OF THE GRAPHS OF THE DIFFERENTLY TREATED PLOTS WITH THE CONTROL (N-TREATED) PLOT [5]

The following comparisons of foliar diagnoses and plant development in different treatments with that in N are based on graphs shown in figure 1 and on data presented in tables 2 and 4. The foliar diagnoses of plants on the plots in tier 1 are in general, and with a sufficient approximation for field experiments, reproduced in the respective duplicate plot in tier 4. Such divergences as exist are the result of soil heterogeneity. The same comments refer also to the foliar diagnosis of the plants of the plots in tier 2 as compared with their duplicates in tier 5.

Comparison of the NPK Plot (1–10) With the N Plot (1–2)

Nitrogen of NPK is lower than that of N throughout the period. The relative steepness of the slopes of the graphs (relative decrease with time) is greater in NPK during the early period (a decline of 1.10 percent for NPK as compared with 0.70 percent for N), indicating a greater evacuation of nitrogen from the leaves of plants growing on the complete fertilizer plot as compared with those on the plot receiving nitrogen alone. This relative excess of export over import is an indication of greater demand by the plant.

The phosphoric acid graphs have neither the same position nor the same form. The graph of NPK is much higher than that of N on July 7—0.58 percent for NPK, as compared with 0.40 percent for N—and, whereas phosphoric acid continues to decline with time in NPK throughout the period, a maximum is shown on July 29 in N. Reference has already been made to this maximum in the plots which did not receive phosphate additions. The decrease from July 7 to August 24 in N is only 0.06 percent, as compared with 0.19 percent in NPK.

The potash graphs also are very unlike in form and position in these plots. Potash is much higher in the leaves of plants growing on the complete fertilizer plot throughout the whole period (the range is from 6.59 to 4.35 percent for NPK and from 3.97 to 1.77 percent for N). In both plots the slope is very steep during the first period, but in NPK a reversal of direction takes place, resulting in a maximum on August 9—an indication that the leaves of the plants growing on the NPK plot have received more potash than has been assimilated by the plant during the second period.

Related to these differences is a much more rapid and luxuriant development of the aerial parts in NPK, in which complete development of the tops has occurred by the end of July. The yield of tubers of NPK is 40 percent greater than in N. These differences are qualitatively reproduced in tier 4.

[5] For the sake of brevity in the discussion that follows, the plot-treatment symbol is used to refer to leaves from plants growing on the plot that received the treatment indicated by the symbol.

Comparison of the PK Plot (1–8) With the N Plot (1–2)

The nitrogen in the leaves of PK is much lower throughout the period than in N. The range is from 4.55 to 2.82 percent for PK and from 5.12 to 3.64 percent for N. The form of the nitrogen graphs of these two plots also is unlike.

It may be noted here that the nitrogen graph of PK is the lowest of all on July 7 and the range corresponds closely with that of K and P.

Phosphoric acid in PK is higher throughout the period and its graph is similar in form to that of phosphoric acid in NPK. No maximum occurs in PK on July 29 as it does in N.

Potash is very much higher in PK than in N. The range is from 6.76 to 5.04 percent for PK and from 3.97 to 1.77 percent for N. At the first sampling the relative values are as wide apart as 6.76 percent and 3.97 percent. The slope of the graph of PK begins to decrease during the second and third periods, showing that evacuation from the leaf is less rapid than in N.

Related to these different foliar diagnoses is a more rapid development of the aerial portion of PK as compared with that of N, especially during the early period. The relative weights of tops per plant in PK and in N on July 25 are 360 g and 162.5 g, respectively. On August 24 the relative yields per plant are 486 g and 274 g, respectively.

It is of interest to note that up to August 24 the yield of tubers per plant for PK exceeds that of NPK,this superiority does not continue as the final yields from NPK are much greater than those from PK.

These differences are qualitatively reproduced in tier 4.

Comparison of the NK Plot (1–7) With the N Plot (1–2)

Nitrogen is somewhat lower and the slope is steeper in NK during the period July 7 to August 24. The range is from 5.10 to 3.26 percent for NK and from 5.12 to 3.64 percent for N.

The phosphoric acid graphs resemble one another in form, but the graph of NK is higher at the first and last sampling dates than that of N. The range is from 0.464 to 0.352 percent for the former and from 0.404 to 0.344 percent for the latter. The maximum already spoken of on July 29 appears in both, although it is higher in N.

Potash is very much higher in NK. The relative ranges are from 6.63 to 5.46 percent for NK and from 3.97 to 1.77 percent for N. The form of the graphs also is very different. Thus, the graph of NK does not descend regularly with time. After the second period the graph of NK reverses direction, indicating that the leaves are receiving more potash than is being assimilated by the plant.

Related to these differences in the foliar diagnosis, the development of the aerial parts and the yield of tubers are much greater in NK than in N. The relative weights per plant of the aerial parts on July 4 are 85.5 g for NK and 59.9 g for N, and on August 24, 386.3 g and 311.3 g, respectively. The yield of tubers on August 24 is 481.2 g for NK and 273.7 g for N.

Comparison of the NP Plot (1–6) With the N Plot (1–2)

Except for an insignificant difference at the first sampling date, nitrogen is lower in NP than in N. The range is from 5.14 to 3.36 percent for NP and from 5.12 to 3.64 percent for N. The relatively greater slope of the graph of NP, especially during the period from

July 7 to July 29, indicates greater utilization of N and at a high level.

Phosphoric acid is nearly 50 percent greater at the first sampling date in NP than in N. The range is from 0.59 to 0.40 percent for NP and only from 0.40 to 0.34 percent for N. A maximum does not occur on July 29 in NP as in N.

Potash is much lower in NP. The range is from 2.53 to 1.08 percent for NP and from 3.97 to 1.77 percent for N. The addition of phosphate, then, has resulted in a decreased absorption of potassium.

The differences shown in the foliar diagnosis have resulted in improved development of the aerial parts and of tubers of NP over those of N. On July 24 the relative weights of tops per plant are 306 g for NP as compared with 162 g for N; and of tubers on August 24, 445 g, as compared with 273 g. The development and yield of the plants growing on plot 1–6, however, are below those of NPK (1–10, 4–8), NK (1–7, 4–11), and PK (1–8, 4–10).

Comparison of the K Plot (1–4) With the N Plot (1–2)

Nitrogen is lower throughout the season in K than in N. The range is from 4.61 to 2.78 percent for K and from 5.12 to 3.64 percent for N, and the slope is greater in K than in N. There is, therefore, more demand on the nitrogen of plants growing on the K plot, which is related to a low level of supply of this element.

Phosphoric acid is somewhat higher in K than in N, although the graphs of both plots are similar in form. The ranges are from 0.424 to 0.370 percent and 0.404 to 0.344 percent in K and N, respectively. Both have a maximum at the second sampling date on July 29. The graphs nearly coincide after the second period.

Potash is very much higher in K than in N. The range is from 6.32 to 5.00 percent in K and from 3.97 to 1.77 percent in N. The a cent of the graph of K during the third period (July 29–August 9) indicates an accumulation of potassium. This phenomenon was noted also in NK (in NPK accumulation occurs during the second period).

Related to these differences in the foliar diagnosis is a superior development of the plants growing on the K plot. The relative weights of tops per plant on July 24 are 230 g for K and 162 g for N, and of tubers on August 24, 481 g and 274 g, respectively.

Although the development of the aerial parts of the plants growing on the K plot is, as in the N plot, relatively slow at first, there is no decrease in the weight of tops as the plant ages as is the case in NPK, PK, NK, NP, and P. The weight of the tubers of PK increases rapidly after July 29. On August 10 the weight of tubers surpasses even that of NPK, but the superiority does not continue.

Comparison of the P Plot (1–3) With the N Plot (1–2)

Nitrogen is much lower throughout the whole period in P than in N. The range is from 4.57 to 2.79 percent for P and from 5.12 to 3.64 percent for N. The forms of the graphs are similar except that the gradient is steeper in P from July 7 to July 29. The nitrogen graph of P coincides very nearly with that of K and with PK.

Phosphoric acid is higher (except on July 29) throughout the period in P. The ranges are from 0.59 to 0.45 percent and from 0.40 to 0.34 percent for P and N, respectively. There is no maximum on July 29 in P.

Potash is lower throughout the whole period in P than in N. The range is from 2.41 to 1.11 percent for P and from 3.97 to 1.77 percent for N.

Related to these differences in the foliar diagnosis is a slightly improved growth of tops of plants growing on the P plots during the early period, after which both nitrogen and potassium become limiting factors. On July 29 the relative weights of the aerial parts per plant are 196 g for P and 162 g for N, and the yields of tubers on August 24 are 302 g and 273 g for P and N, respectively. The P plot shows the poorest development of tops of any of the plots after July 29, but the yield of tubers, as already indicated, is higher than in the N plot.

RELATION OF DEVELOPMENT TO FOLIAR DIAGNOSIS OF PLANTS RECEIVING DIFFERENT PROPORTIONS OF N, P_2O_5, AND K_2O IN COMPLETE FERTILIZERS AND THOSE RECEIVING THE STANDARD APPLICATION

The development of plants and the composition of the fourth leaf at the different sampling dates in plots receiving different proportions of N, P_2O_5, and K_2O in complete fertilizers are shown in tables 5 and 6, and in figure 2.

TABLE 5.—*Development of tops and of tubers from potato plants receiving different proportions of nitrogen, phosphoric acid, and potash in complete fertilizers*

[Fresh weights per plant]

Treatment	Tier No.	Plot No.	Portion	July 4	July 25	August 10	August 24
				Grams	*Grams*	*Grams*	*Grams*
0.5(NPK)	2	14	Aerial	87.9	267.5	311.3	372.5
			Tubers	19.6	110.0	256.2	487.5
	5	4	Aerial	89.4	345.0	296.3	311.3
			Tubers	21.6	160.0	290.0	462.5
2(NPK)	2	16	Aerial	200.3	872.5	945.0	492.5
			Tubers	24.5	205.0	492.5	611.0
	5	2	Aerial	120.6	407.5	515.0	540.0
			Tubers	22.4	132.5	281.3	450.0
(1.5 N)PK	2	4	Aerial	183.3	543.8	635.0	648.8
			Tubers	29.2	157.5	355.0	583.7
	5	14	Aerial	114.4	341.3	407.5	375.0
			Tubers	23.5	113.7	212.5	381.2
N(1.5 P)K	2	8	Aerial	119.6	387.5	448.8	502.9
			Tubers	21.8	163.8	392.5	494.2
	5	10	Aerial	140.8	520.0	517.5	532.5
			Tubers	25.3	190.0	330.0	565.0
NP(1.5 K)	2	12	Aerial	124.9	425.7	457.5	582.5
			Tubers	19.7	137.2	392.5	621.3
	5	6	Aerial	103.0	348.8	370.0	----------
			Tubers	20.0	176.2	256.3	----------
Manure	1	15	Aerial	251.9	446.3	501.3	692.5
			Tubers	31.1	152.5	308.7	753.8
	4	3	Aerial	125.4	545.0	525.0	635.0
			Tubers	22.1	173.0	313.8	392.5

COMPARISON OF THE 0.5 (NPK) PLOT (2–14) WITH THE NPK PLOT (1–10)

Nitrogen is lower throughout the period in 0.5 (NPK) than in NPK. The range is from 4.84 to 2.84 percent for 0.5 (NPK) and from 4.98 to 3.14 percent for NPK.

Phosphoric acid also is lower in 0.5 (NPK) except at the second and the last periods. The range is from 0.52 to 0.42 percent for 0.5 (NPK) and from 0.58 to 0.39 percent for NPK.

TABLE 6.—*Nitrogen, phosphoric acid, and potash content on different dates of the fourth leaf of potato plants receiving different proportions of these minerals in complete fertilizers, expressed on a dry-weather basis*

Treatment	Tier No.	Plot No.	Mineral nutrient	July 7	July 29	August 9	August 21
				Percent	*Percent*	*Percent*	*Percent*
0.5 (NPK)	2	14	N	4.84	3.77	3.34	2.84
			P_2O_5	.520	.528	.466	.418
			K_2O	6.108	4.379	4.031	3.920
	5	4	N	4.34	3.57	3.12	2.75
			P_2O_5	.524	.486	.454	.400
			K_2O	6.705	4.379	4.310	4.162
2 (NPK)	2	16	N	5.23	4.04	3.87	3.30
			P_2O_5	.682	.562	.500	.354
			K_2O	8.771	5.920	6.124	6.453
	5	2	N	5.03	4.08	3.76	3.35
			P_2O_5	.650	.509	.454	.368
			K_2O	7.521	5.50	5.658	5.775
(1.5 N) PK	2	4	N	5.23	4.00	3.98	3.16
			P_2O_5	.614	.488	.464	.356
			K_2O	6.780	5.040	4.534	4.302
	5	14	N	4.98	4.26	3.76	3.23
			P_2O_5	.528	.446	.430	.348
			K_2O	6.814	5.744	5.704	5.310
N (1.5 P) K	2	8	N	4.84	3.72	3.46	2.86
			P_2O_5	.710	.564	.482	.350
			K_2O	6.969	4.914	4.934	4.670
	5	10	N	4.84	3.90	3.40	2.96
			P_2O_5	.658	.608	.576	.404
			K_2O	6.678	5.031	4.883	4.961
NP (1.5 K)	2	12	N	4.86	4.19	3.71	2.82
			P_2O_5	.594	.522	.513	.394
			K_2O	7.798	5.767	5.782	5.996
	5	6	N	4.54	3.71	3.26	3.01
			P_2O_5	.554	.502	.444	.382
			K_2O	6.794	5.124	4.653	5.310
Manure	1	15	N	4.65	3.80	3.79	3.28
			P_2O_5	.688	.608	.564	.448
			K_2O	7.550	5.128	5.155	4.658
	4	3	N	4.84	4.04	3.58	3.45
			P_2O_5	.750	.604	.502	.472
			K_2O	7.248	5.116	4.988	5.601

Potash is lower except in the second period in 0.5 (NPK). The range is from 6.11 to 3.92 percent for 0.5 (NPK) and from 6.59 to 4.35 percent for NPK. The forms of the graphs are different. No accumulation of potassium occurs after the second period in 0.5 (NPK) as in NPK.

Corresponding to these differences in the foliar diagnosis is a much slower development of the aerial parts of 0.5 (NPK) during the early part of the season. The yield of tubers of 0.5 (NPK) is 8 percent below that of NPK.

COMPARISON OF THE 2 (NPK) PLOT (2–16) WITH THE NPK PLOT (1–10)

Nitrogen is higher throughout in 2 (NPK). The range is from 5.23 to 3.30 percent for 2 (NPK) and from 4.98 to 3.14 percent for NPK. The forms of the graphs have the same characteristics in both plots.

Phosphoric acid also is higher in 2 (NPK) except during the last period of growth. The range is from 0.68 to 0.35 percent for 2 (NPK) and from 0.58 to 0.39 percent for NPK. The relative steepness of the slope of the 2 (NPK) graph shows that greater evacuation of phosphorus has taken place, indicating better assimilation and at a high level.

Potash is very much higher throughout the whole period in 2 (NPK). The range is from 8.77 to 6.45 percent for 2 (NPK) and from 6.59 to 4.34 percent for NPK.

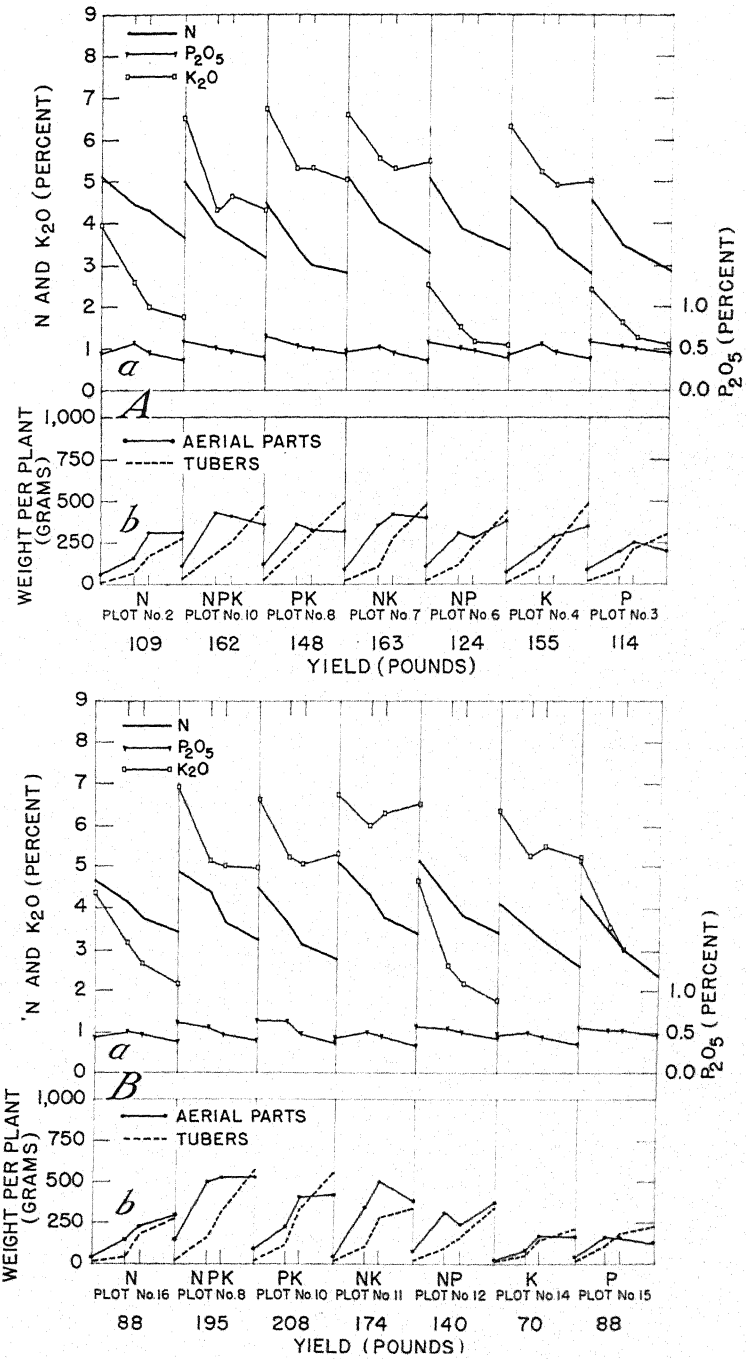


FIGURE 2.—Nitrogen, phosphoric acid, and potash content of the fourth leaf (dry foliage) of potato plants at four periods of sampling (*a*), and the weights of aerial parts and of tubers at corresponding dates (*b*) from plots receiving different proportions of these minerals in complete fertilizers: *A*, Tiers 1 and 2; *B*, tiers 4 and 5.

The potash content of the leaves of 2 (NPK) is higher than that of leaves from any other plot. But it cannot at the present stage be determined whether this high potassium content in the leaves of plants from plot 2–16 has resulted in any injury to development or yield. It is possible that the rapid loss of weight of tops referred to in the next paragraph is due to this cause. The fact that this loss does not occur in 5–2 where the potassium content is lower lends confirmation to this view. But what would be the limit of potassium absorption above which injury would be shown and where the boundary of cessation of buffer action lies are not disclosed by this experiment.

After July 29 both graphs reverse direction, but whereas that of 2 (NPK) continues its ascent that of NPK declines. It is significant that this accumulation of potassium occurs among the relatively high yielding plots only.

Related to these differences in the foliar diagnosis is a very luxuriant growth of tops in 2 (NPK), followed by a rapid loss in weight after August 10. The yield of tubers has been increased 28 percent.

Comparison of the (1.5N) PK Plot (2–4) With the NPK Plot (1–10)

Nitrogen is a little higher in (1.5N) PK than in NPK. The range is from 5.23 to 3.16 for the former and 4.98 to 3.14 percent for the latter. The forms of the graphs, however, are much alike.

Phosphoric acid is slightly higher in (1.5N) PK. The range is from 0.61 to 0.36 percent for (1.5N) PK and from 0.58 to 0.39 percent for NPK. The forms of the graphs are somewhat similar.

Potash is somewhat higher throughout the first two periods in (1.5N) PK than in NPK. The forms of the graphs are quite different.

Related to these differences in the foliar diagnosis, the vegetative growth of plants on the (1.5N) PK plot far surpasses that of plants on the NPK plot throughout the period, and the weights of tubers of the former on August 24 are greater than those of the latter.

Comparison of the N (1.5P) K Plot (2–8) With the NPK Plot (1–10)

Nitrogen is slightly lower throughout the period in N (1.5P) K. The range is from 4.84 to 2.86 percent for this plot and from 4.98 to 3.14 percent for NPK. The forms of the graphs, however, are similar.

Phosphoric acid is very much higher in N (1.5P) K during the first two periods, after which greater utilization in N (1.5P) K causes the graft to decend below that of NPK. The range is from 0.71 to 0.35 percent for this treatment and from 0.58 to 0.39 percent for NPK. The relative steepness of the slope of N (1.5P) K indicates a better utilization of phosphorus than in NPK.

Potash is higher throughout the period in N (1.5P) K. The range is from 6.97 to 4.67 percent, as compared with 6.59 to 4.35 percent for NPK. The forms of the graphs are somewhat similar, however.

Related to these differences in the foliar diagnosis is the fact that, although from July 4 to July 25 the vegetative growth and development of tubers are nearly identical in N (1.5P) K and NPK, vegetative growth continues in the plants growing on the former plot whereas a loss of weight is shown in those on the latter. The yield of tubers is 8 percent greater in N (1.5P) K than in NPK.

Comparison of the NP(1.5K) Plot (2–12) With the NPK Plot (1–10)

Nitrogen is a little lower during the first and fourth periods in NP(1.5K), ranging from 4.86 to 2.82 percent, as compared with 4.98 to 3.14 percent for NPK, but the forms of the graphs are similar.

The phosphoric acid graphs almost coincide; they range from 0.59 to 0.39 percent and from 0.58 to 0.39 percent for NP(1.5K) and NPK, respectively.

Potash is very much higher in NP(1.5K) without, however, reaching the level attained in 2(NPK). The ranges are from 7.80 to 6.00 percent for NP(1.5K) and from 6.59 to 4.35 percent for NPK. The forms of the graphs also are different. Potash accumulates in NP(1.5K) after the second period.

Related to these differences is superior development of the aerial parts and tubers in NP(1.5K).

Comparison of the Manure Plot (1–15) With the NPK Plot (1–10)

Nitrogen is lower on the manure plot at the first sampling date and higher at the last date than in NPK; the ranges are from 4.65 to 3.28 percent and from 4.98 to 3.14 percent, respectively.

Phosphoric acid is much higher throughout the period in the leaves from the manure plot; the ranges are from 0.69 to 0.45 percent and from 0.58 to 0.39 percent for manure and NPK, respectively.

Potash is higher at each date of sampling in the manure plot. The range is from 7.55 to 5.66 percent for manure and from 6.59 to 4.35 percent for NPK. Potash accumulation occurs during the last period (August 24) in the manure plot.

Related to these differences is a much greater development of the plants on the manure plots which is noticeable even on July 7 and which, with the exception of the second period, continues throughout the period. The yields of tubers per plant are 754 g in the manure plot and 466 g in NPK on August 24.

Note on the Development of the Plants

The development graphs of all plots are regularly progressive and show no indications of any interfering accidental factors outside of those studied. Plants on duplicate plots have not always developed in like manner. Between some of the duplicates there are marked differences in both aerial parts and tubers, suggesting the influence of soil on the action of fertilizers.

SUMMARY

Foliar diagnosis is defined as the composition, with respect to the dominant elements, of a leaf from a definite position (physiological age) on the stem at the moment of sampling. The foliar diagnosis of any particular species during a given season's growth cycle will consist, therefore, in a sequence of chemical states in the selected leaf as determined by periodic analysis. The composition with respect to nitrogen, phosphoric acid, and potash is based on the dry material of the leaf without taking into consideration either the weight or the number of leaves sampled.

The nitrogen, phosphorus, and potassium content of leaves of the same physiological age, sampled periodically, from potato plants

grown on plots receiving different fertilizer treatments with respect to nitrogen, phosphoric acid, and potash have been studied in relation to the nature of these treatments and to the development of the plants. The characteristics are fully described.

The addition of each of the dominant nutrient elements to the soil increased the content of the element in the leaves sampled, as compared with the content in leaves without applications of the element, whether the element was applied alone or in combination with one or both of the other elements. Utilization of any nutrient, however, was profoundly influenced by the presence in the fertilizer of other nutrients, as illustrated by accumulation of potash in leaves from plots receiving potash fertilizer but with insufficient nitrogen or phosphorus.

The content of nitrogen in the leaves is not related to the development of the plants; low phosphorus content and low utilization of this element are, however, associated with unsatisfactory development. Equilibrium between nitrogen and phosphorus throughout the season is associated with good development, as is also a maintained rapid utilization of potassium at a high level.

LITERATURE CITED

(1) LAGATU, HENRI, and MAUME, LOUIS.
[1930]. LE DIAGNOSTIC FOLIAIRE DE LA POMME DE TERRE. PREMIER MÉMOIRE Montpellier Ecole Nat. Agr. Ann. 20: 219–281, illus.

(2) ——— and MAUME, L., with the collaboration of CROS, LUCIENNE; DELOCHE, LUCIE; and FABREGUETTES, MARCELLE.
n. d. LE DIAGNOSTIC FOLIAIRE DE LA POMME DE TERRE. DEUXIÈME MÉMOIRE. Montpellier Ecole Nat. Agr. Ann. 22: [50]–158, illus.

(3) MACK, W. B.
1927. FERTILIZATION OF TRUCK CROPS IN ROTATION. Pa. Agr. Expt. Sta. Bull. 210, 31 pp.

(4) MACK, WARREN B., and TUTTLE, ALDEN P.
1932. THE RELATIONS AMONG FERTILIZER TREATMENT, SOIL MOISTURE, ORGANIC MATTER, AND YIELD OF VEGETABLE CROPS. Jour. Amer. Soc. Agron. 24: 182–202, illus.

(5) SANDROCK, WILHELM.
1931. KRITISCHE BETRACHTUNGEN ZUR BLATTDIAGNOSE NACH LAGATU BEI DER KARTOFFEL UNTER BESONDERER BERÜCKSICHTIGUNG DES IM BODEN DURCH DIE KEIMPFLANZENMETHODE ERMITTELBAREN WURZELLÖSLICHEN NÄHRSTOFFVORRATES. Ztschr. Pflanzenernähr., Düngung u. Bodenk. (B) 10: 188–201.

(6) THOMAS, WALTER.
1937. FOLIAR DIAGNOSIS: PRINCIPLES AND PRACTICE. Plant Physiol. 12: 571–599.

(7) VINET, E., and LEMESLE, M.
1930. ESSAIS SUR LES ENGRAIS APPLIQUES A LA VIGNE. Bull. Mens. Soc. Indus. et Agr. d'Angers 101: 81–97.

OBSERVATIONS ON MUSKRAT DAMAGE TO CORN AND OTHER CROPS IN CENTRAL IOWA[1]

By PAUL L. ERRINGTON

Research assistant professor, Iowa Agricultural Experiment Station

INTRODUCTION

Despite the value of the muskrat (*Ondatra zibethica*) as a fur bearer, popular sentiment regarding the species is not always favorable. Many people believe that it causes economic losses because of its feeding or digging proclivities. In central Iowa, one of the chief sources of complaint against the muskrat is its occasionally destructive foraging in cultivated fields. Damage to farm crops by wild animals is likely to be overestimated, however, when it becomes conspicuous. It is hoped, therefore, that the present paper, which is based upon a study of muskrat food habits in intensively cultivated land, may help to a clearer evaluation of at least some types of local situations.

METHODS OF INVESTIGATION

Basic data on the food habits of muskrats were obtained from the summer of 1934 to the winter of 1937–38, largely through field observations along watercourses within a radius of 20 miles from Ames, Iowa, or from Story County and the neighboring counties of Hamilton, Boone, Dallas, and Polk; supplementary observations were also made in Wapello and Jefferson Counties in the southeastern part of Iowa and in Clay and Palo Alto Counties in the northwestern part.

Representative muskrat habitats [2] were chosen for study. These varied in nature from brooks and small drainage ditches to marshlands and the fairly large Des Moines River. Stream habitats under ordinarily intensive observation were visited at intervals sufficient to detect evident feeding trends, influence of seasonal changes in vegetation and water levels, and the comparative role of availability of food and individual preference in determining the feeding routine of muskrats. When events of exceptional interest or significance were found to be taking place, specific habitats were visited daily or several times a week for necessary periods; otherwise, habitats were seldom visited more than once a week. Pressure of work elsewhere or conditions unfavorable for field study sometimes necessitated temporary neglect of important habitats, but, on the whole, the same local areas were kept under observation during the 4 years of the investigation.

The general procedure was to take notes on vegetation cut or eaten by muskrats on stream banks, on the food debris piled on or scattered around feeding places in or near the water, and on the material carried to burrows which were sometimes exposed by receding water or caving

[1] Received for publication March 18, 1938; issued September 1938. Journal Paper No. J–532 of the Iowa Agricultural Experiment Station; project No. 498. The work herein recorded was conducted by Iowa State College in cooperation with the Bureau of Biological Survey, U. S. Department of Agriculture, the American Wildlife Institute, and the Iowa Conservation Commission.

[2] ERRINGTON, PAUL L. HABITAT REQUIREMENTS OF STREAM-DWELLING MUSKRATS. North Amer. Wildlife Conf. Trans. 2:411–416. 1937.

of the ground so that one could look inside with the aid of a flashlight. While this ex post facto "reading of sign" doubtless was accompanied by a certain amount of overlooking if not misinterpretation of field evidence, the accuracy of the method was checked whenever possible by other means. Occasionally, the examination of muskrat feces or stomach contents provided information of value, but most useful of all in clarifying dubious interpretations of "sign" was direct observation of the muskrats themselves.

Many times, muskrats were watched from distances of 15 to 40 feet when feeding or obtaining food. Details of procedure in selecting, cutting, digging, carrying, and eating of food items were recorded in field notes, and the sites of activity were often carefully scrutinized. On exposed feeding grounds still in use during the months of cold weather, snow trails of individual muskrats were followed and studied.

FORAGING TENDENCIES OF THE MUSKRAT

Muskrats, being animals of semiaquatic habitats, typically cut or feed upon aquatic or weedy vegetation growing in the vicinity of their retreats, but they may at times forage on higher ground some distance away.

Considerable feeding may occur in pasture growths of bluegrass and white clover, in timothy, sweetclover, and alfalfa hayfields, and in fields of small grain, but even when muskrat trails to such places show regular traffic it is often hard to find much evidence of damage. Ordinarily the animals forage lightly over an area of several hundred square feet, but, on the other hand, places may be found near the edges of fields where muskrats have cut away entire stands of medium weight vegetation from irregular areas ranging in size from less than a square foot to perhaps 15 or 20 square feet. Muskrats have also been known to make inroads on garden vegetables, but damage to farm crops in the areas with which this paper deals seems virtually synonymous with damage to corn.

Damage to corn may, too, be negligible or it may be locally severe. Much depends upon the accessibility of the cornfields to resident muskrats and the habits that the animals develop. Muskrats do not like to leave the relative safety of water very far behind, and this automatically limits their zone of heaviest potential damage to the nearest corn rows. Once a muskrat learns to utilize corn plants or ears, it usually prefers these foods to others currently available, and to obtain them may expose itself to material hazards. During the winter of 1934–35, one farm dog was reported to have killed several muskrats that had been traveling a distance of over 200 yards from a creek to a corncrib. When such overland journeys are made to a specific destination, the muskrats tend to establish crooked but well-used trails which afford them the concealment or protection of roadside ditches, low-hanging trees, brush piles, or similar cover. Nevertheless, corn rows within 15 yards of the water are most likely to receive regular visits from muskrats.

SEASONAL CHANGES IN HABITS OF CORNFIELD-FEEDING MUSKRATS

In winter, Iowa stream-dwelling muskrats are commonly confined rather closely to their burrows and associated water, ice shelves, and snowdrifts. With the passing of cold weather, early spring visits to

cornfields may be made either accidentally by wandering or venturesome individuals or seemingly with purposeful intent on the part of individuals that presumably have been there before. Ear corn may be found on the ground in some quantity—even after a field has been pastured and trampled by livestock—but less nutritious corn debris, including dry stalks, leaves, and husks, may also be carried to spring feeding places and eaten.

During the next few weeks, the natural plant growth near the water furnishes more convenient and attractive food, and there may be no sign of renewed activity in the cornfields until about the first week of July or later. By this time, the corn plants in central Iowa may be 3 or 4 feet high, and seem considerably more succulent and palatable than much of the natural vegetation available to the muskrats. The stalks are usually cut between 3 and 12 inches from the ground and, in places thought to be visited by lone muskrats, the rate of cutting has been only one or two per day. The whole upper part of the cut plant may be carried to a regular feeding place at the water's edge, or much feeding may be done in the cornfield at the site of cuttings. The canes seem especially relished by the muskrats and the upper leaves least so.

The corn plants that are about 6 feet high by the middle of July are cut in limited amounts, and haphazard or desultory gnawing may weaken a number of others and eventually cause them to fall. The interest of the muskrats in vegetative portions of corn plants sometimes diminishes in late July, and prior to the roasting-ear stage there may be scant evidence of current activity in the cornfields.

Roasting ears in the second half of August, however, may constitute a major attraction. While many muskrats take ears from leaning or fallen stalks or reach up as high as 15 inches for them, there may be wholesale cutting of stalks for ears by other individuals or groups of individuals. Commonly, the ears are cut loose at the base and carried away, though an occasional ear may be eaten from below as it hangs from its stalk. Aside from ears dropped in trails and in the water in front of the entrances of the burrows, there may be little external sign of most of the ears taken; but caved-in sections of burrows and similar retreats may reveal ears and mixed corn debris, so it is probable that the bulk of the material goes underground for safer consumption if not for actual storage. Ears found about muskrat retreats at this season, whether in plain sight on land or in water or retrieved from burrows or tile openings, show all stages of feeding, from specimens with most of the husks intact to those with not only husks and kernels gone but also large pieces of cob.

With the ripening of the corn in September, cutting of stalks for ears may persist locally, but according to observational data in hand, on a decreased scale. There may be continued heavy or accelerated traffic into the cornfields for ears on fallen stalks or lying free on the ground. From ripening time until the water courses freeze over, the most general foraging in cornfields is noted. Ripened ears, like those in the roasting-ear stage, are usually taken into the burrows. The evidence suggests that corn ears stored or accumulated in the burrows comprise a very important item in the winter diet of many muskrats, even after the material becomes soggy and stale.

After "freeze-up," some muskrats, for no known reason, persist in visiting cornfields long past the time that other muskrats have

ceased to come out, or they resume outside activities during thaws. Others, such as those living in burrows exposed by the drying up of marshes and stream beds, may through necessity and regardless of the weather, forage daily in the open, in which event cornfields may be particularly inviting to them. Still others may wander about the countryside, establishing themselves in farmyards, corncribs, corn shocks, or simply living where they can; muskrats thus abroad in midwinter find little in corn fields except dry stalks rejected by cattle, but they eat these in the absence of anything better.

During dry years, muskrats evicted from their accustomed habitats may actually improvise winter quarters in the cornfields. These quarters vary from flimsy temporary nests lined with dry grass and husks to large dry-land lodges constructed of stalks, weeds, mud, and other portable though substantial materials. One farmer reported finding about a bushel of ear corn in one cornfield habitation that he opened.

EVALUATION OF AN EXTREME INSTANCE OF MUSKRAT DAMAGE TO CORN

The most conspicuous damage from muskrat feeding that was noted during the investigation occurred along a half-mile stretch of drainage ditch southwest of Story City, Iowa.[3] Since destructive raiding into adjoining cornfields appeared to be a regular annual phenomenon, the ditch and environs were kept under careful observation throughout 1937.

In that year corn was planted in three fields bordering almost one entire side of the ditch; on the opposite side of the ditch was a closely grazed pasture, a field of mixed oats and barley, and a potato field. The ditch itself was about 5 feet wide at the bottom and about 8 feet below the level of most of the surrounding land, but it often contained only an interrupted series of shallow puddles without water enough for a steady flow. The dirt from excavation had been piled on both sides of the ditch in parallel ridges. The ditchbanks were fairly well protected from grazing by livestock, and the vegetation was principally of grasses with growths of Compositae, Polygonaceae, Cyperaceae, and miscellaneous weeds.

The spring breeding population of muskrats was not determined with certainty, but there were four main foci of activity along the half mile of ditch at two of which young animals or tracks and feces of young animals could be seen later in the season. Young muskrats appear to remain quite close to their natal localities after weaning,[4] so a resident breeding population of two, and perhaps four, pairs of adults seemed probable. Carcasses of 39 muskrats trapped in November 1937 from the ditch, and mostly from the half mile under observation, proved to be of those of 3 adult males, 2 adult females, and 34 young of the season.[5] By November, the population along the half mile of ditch was largely concentrated in two sets of burrows; according to the trappers, the more extensive set yielded about 20 muskrats without having been completely trapped out and the other

[3] NE¼ sec. 22, T. 85 N., R. 24 W., Story County.

[4] ERRINGTON, PAUL L., and ERRINGTON, CAROLYN STORM. EXPERIMENTAL TAGGING OF YOUNG MUSKRATS FOR PURPOSES OF STUDY. Jour. Wildlife Management 1 (3-4):49-61, illus. 1937.

[5] The Iowa muskrat investigations have brought forth no evidence of young muskrats becoming sufficiently mature to breed in the same season in which they are born. See the following: ERRINGTON, PAUL L. THE BREEDING SEASON OF THE MUSKRAT IN NORTHWEST IOWA. Jour. Mammal. 18:333-337, 1937; and the reference cited in footnote 4.

yielded a total of 7. This should indicate a pretrapping fall population close to 40 individuals, made up for the most part of the adults, and increase of from 2 to 4 pairs, if the sex and age ratios of the ditch-caught muskrats are representative of the animals resident along the half mile under observation. Incidentally, the ratio of young animals to adults given above is the highest recorded in the course of 4 years' study.

From March 7 to 16, 1937, evidence was found that muskrats had visited cornfields nearby and fed on ear corn at the water's edge. Some feeding on ear corn was noted as late as April 14, after which the animals seemed to find the cornfields less profitable foraging than the vegetation-grown ditchbanks. Thereafter, no sign of renewed activity in cornfields was detected until the new corn plants were of substantial size.

By July 14 and 15, considerable evidence of muskrats cutting down corn plants could be seen in the three cornfields bordering the half mile of ditch. At this time, the cutting was judged to have started more than a week previously in some places and less than a week in others. A counted total of 144 corn plants had been cut up to July 15. Between July 15 and 30, stalks were being cut at rates ranging from 6 to 11 per day and averaging about 2 per day for each of the four main foci of activity.

A marked increase of foraging in the cornfields occurred in mid-August and this had reached its apparent height by August 20. It is not clear whether this was due to the attractiveness of the ear corn, now at the roasting-ear stage, or to the adults having been joined in their foraging by the larger of the young. Some of the young were doubtless between 3 and 4 months old and nearly as large as small adults. In July, young animals were observed feeding chiefly on natural vegetation in or near the water, but there is no reason to believe that the larger ones were not venturing out to help themselves to the roasting ears. The technique of the muskrats in procuring the ears, however, seemed to be that of strong and experienced animals.

Most of the roasting ears were taken from the four main feeding areas where there had been cutting of corn plants earlier in the summer. In two of these, the muskrats took ears principally from leaning or fallen stalks and did little cutting; in the other two, cutting of stalks for ears was noticeable on an extensive scale and, on August 25, at the rate of about 40 per day. The total number of cut stalks counted up to August 20 was 1,304.

No thorough examinations of the affected cornfields were made from August 25 to September 19, but by the latter date, cutting of stalks for ears had greatly decreased with the ripening of the corn about 2 weeks before. Stalks evidently cut since ripening totaled 115. These added to an estimated 40 per day for perhaps 15 days subsequent to August 20, plus those cut before August 20, would give something like 2,000 cut stalks by September 19.

From this time on to November 4, the cutting and foraging of most of the muskrats was centered in an area in the corner of one of the cornfields. The area of intensive exploitation increased from 222 square yards on September 19 to 718 square yards by November 4; the few stalks left standing were estimated to be about the same in number as those cut outside, so, for practical purposes, the corn in the area cut over should be roughly the equivalent of that taken by the

muskrats. The increase of 496 square yards in cut area amounts to the destruction of 364 hills. One hundred eighty-two hills averaged 3.1 stalks, and this would give a total of 1,128 stalks cut by the muskrats since September 19, or an average of 24.5 per day for 46 days. Elsewhere along the half mile of ditch, muskrats visited the cornfields principally for ears on the ground, including those overlooked by men picking corn.

Subsequent damage was ascertained with less accuracy. The trapping season opened November 10, and the muskrats using the trails were soon caught. A few days later, the weather turned cold enough to put an end to the cornfield visits of the remaining muskrats for the rest of the fall. It seems reasonable to assume that the muskrats averaged 8 days of foraging after November 4. On the pro-rata basis of 24.5 stalks cut per day, about 188 additional stalks would be cut for ears before the death of the animals or the completion of the corn picking terminated destructive types of feeding activity in the cornfields.

To recapitulate: A total of about 3,300 cornstalks cut from July to the middle of November 1937, constituted the recognized muskrat damage to corn in three fields adjacent to the half mile of drainage ditch. Two hundred stalks examined at random in the fields bore 171 ears (including 18 nubbins), which would make the 3,300 cut cornstalks equivalent to the loss of over 2,800 ears of corn; at the average weight of 7.35 ounces per ear obtained from a random sample of 81 ears, 2,800 ears would represent a loss approaching 18.4 bushels of corn chargeable to the resident muskrat population.

The price of 1937 corn in Story County was $0.39 per bushel on November 15,[6] and fur buyers were offering locally about $0.60 flat rate for muskrat pelts. While the actual sums received from both corn and muskrat pelts would vary with circumstances and with the judgment of the sellers, at the above ratio of prices the $16.20 fur value of the 27 muskrats trapped is over twice the $7.18 known corn loss.

Even if one arbitrarily added $3 or $4 to the computed corn damage to cover the loss of ears taken by muskrats without cutting stalks—which really did not appear to have economic significance in this instance—the total damage to the corn would still be lower than the fur returns from the half mile of ditch. It is, of course, true that the farmers losing corn are not necessarily the persons to profit from the sale of the trapped pelts, though to do so is often within their choice.

DISCUSSION

During 1937, in particular, a concerted effort was made to evaluate muskrat damage to farm crops in the fields bordering about 11 miles of watercourses, exclusive of minor windings of channels. Dry weather in late summer and early fall evicted muskrat populations from some stretches of stream bed, but it is not thought that this reduced the number of animals enough to result in appreciable lessening of foraging in cornfields still accessible. On the contrary, the drought may be said to have intensified muskrat activity at increasing distances from their regular quarters.

[6] County agricultural agent reports.

Observations were made in 26 fields of corn within convenient reach of habitats occupied by muskrats in 1937. Of these, only the three adjacent to the half mile of drainage ditch described in this paper consistently suffered damage, but, in one other case, the autumn traffic for ears on fallen stalks was such that perhaps 30 or 40 ears could be seen at one time on the river bottom in front of a well-used system of burrows.

Twelve of the twenty-six cornfields showed evidence of light or very moderate use, and 10 were apparently not used at all by muskrats. Moreover, the great majority of central Iowa cornfields are not nearly so accessible to muskrats as the 26 under observation.

As a rule, foraging by muskrats occurs year after year in much the same local areas. Fields intersected by drainage ditches are particularly susceptible to damage by muskrats living in the vicinity; fields surrounding marshes are least troubled, possibly because of the abundance of favored foods usually present in natural marshy habitats.

SUMMARY

Data on food habits of muskrats in central Iowa were gathered from the summer of 1934 to the winter of 1937–38. Much the same areas were kept under observation during the entire period of study, and these were representative of intensively cultivated farm land adjacent to muskrat stream habitats of various types. Damage to farm crops through the foraging of muskrats was confined almost exclusively to corn in fields close to watercourses.

The heaviest damage to corn recorded during the investigation occurred in three fields adjacent to a half mile of drainage ditch in Story County. The situation here was observed in some detail throughout 1937, and the visible loss in yield because of muskrats was appraised at $7.18 (equivalent of 18.4 bushels at $0.39 as of November 15). The current market value of the muskrat pelts taken by trappers from corresponding parts of the ditch was $16.20 (27 pelts at $0.60 flat rate).

Of 26 cornfields bordering about 11 miles of muskrat-occupied watercourses in 1937, only 1, in addition to the 3 near the drainage ditch, showed clear evidence of damage; the other 22 either were not used at all by muskrats or were used so slightly that the losses were immaterial.

TOXIC AND REPELLENT PROPERTIES OF SOME INORGANIC AND ORGANIC COMPOUNDS AND MIXTURES IN GRASSHOPPER BAITS [1]

By CHARLES H. RICHARDSON, *research professor*, and EDWIN J. SEIFERLE, *research assistant, Entomology and Economic Zoology Section, Iowa Agricultural Experiment Station* [2]

INTRODUCTION

This investigation forms a part of a project which has continued in Iowa for a number of years, some of the results having been published in 1932 (*9*) [3] and 1933 (*10*). The practical objective has been a poison less toxic to human beings and domestic animals than sodium arsenite yet equally effective on grasshoppers, readily available, and low in cost. Attention has also been given to repellent action and to other phenomena of toxicological interest, for it is recognized that a compound even mildly toxic or repellent to such resistant insects as grasshoppers might be valuable in the control of less resistant species.

Previous studies in this series were concerned with the rather precise estimation of the dosage of a few poisonous compounds; in this investigation, an attempt has been made to survey the toxic and repellent properties of a much larger number of inorganic and organic chemicals.

The experiments were made at Ames, Iowa, during the period from June 1 to August 12, 1937.

MATERIALS

One hundred and ten compounds and mixtures were tested in bran-water baits during the season. Unless otherwise stated in table 1, all the inorganic compounds were of chemically pure or reagent grade. Some of United States Pharmacopoeia grade are so indicated. Most of the organic chemicals were of the highest grade obtainable from reliable manufacturers. Some, however, were of a technical grade as shown in the table. The nature of the mixtures is described briefly in footnotes.

The bran, which formed about half the weight of the finished bait, was a clean, flaky, commercial product milled from wheat.

The test insects were adults of the lesser migratory grasshopper (*Melanoplus mexicanus mexicanus* Saussure). They were captured in western and southern Iowa, kept in large cages, and fed daily on fresh corn foliage. The insects to be used in the tests were held in the laboratory overnight without food.

---

[1] Received for publication March 18, 1938; issued September 1938. Journal Paper No. J-536 of the Iowa Agricultural Experiment Station; project No. 137.

[2] The writers are indebted to L. E. Mills of the Dow Chemical Co., to Dr. J. L. Horsfall of the American Cyanamide Co., to Dr. R. C. Roark of the Bureau of Entomology and Plant Quarantine, and to Dr. W. H. Tisdale of DuPont de Nemours & Co. for certain chemicals; and to Jean Austin for able assistance.

[3] Italic numbers in parentheses refer to Literature Cited, p. 432.

METHOD

The bait consisted of 10.0 g of bran, 9.8 cc of distilled water, and 0.2 g of the compound or mixture, the concentration of the latter ingredient being therefore 1 percent by weight. Insoluble solids, if not already in a finely divided condition, were ground to a fine powder before they were incorporated in the bait. Water-soluble substances were dissolved in the water before it was mixed with the bran. The bait was held in covered containers which were opened only when a quantity of it was removed for the tests.

The method of administering the bait was modified somewhat from that previously described (*9*), for it was found that *Melanoplus m. mexicanus* was more active than the species hitherto employed, frequently upsetting the bait containers when attempts were made to feed the insect in large moist chambers. The modification consisted in weighing about 0.5 g of the moist bait in a small, tared container made of paraffined paper with a hole in one edge by which it was suspended from a hook on the beam of the balance. The container was thrust toward the stoppered end of a large glass tube which was always maintained in a horizontal position. A narrow strip of screen wire provided the tube with a floor which caught and held the bait particles sometimes spilled by the insect. A previously weighed grasshopper was carefully introduced into the open end of the tube which was then closed with a cork stopper. A wad of moist cellucotton, fastened to this stopper, supplied moisture to the air in the tube. The change in moisture content of the bait during the feeding period was determined, and found to be within the limits of precision of the method. When feeding ceased, the insect was removed to a small screen cage where it was observed at intervals until death; or, if apparently unaffected, for a period of 72 hours.

Two standard baits were administered throughout the experimental period to grasshoppers taken from the same populations that furnished individuals for the other tests. The standard baits were: (1) A bran-water bait (10.0 g of bran and 10.0 cc of water) on which 50 insects were fed during the period; (2) sodium arsenite bait (10.0 g of bran and 10.0 cc of an aqueous solution containing 0.2 g of As_2O_3 as NaH_2AsO_3) fed to 66 insects during the same period. The latter mixture contained approximately the same concentration of sodium arsenite calculated as As_2O_3 as that employed in Iowa during the grasshopper-control campaign of 1937. Molasses was not used in these baits; it is now practically excluded from the baits employed in large-scale poisoning in the Middle West.

A test consisted of 10 grasshoppers fed individually, of which 5 were males and 5 females in order to equalize possible sex differences. Since about 1 in each sample of 10 control insects fed the bran-water standard died from natural causes,[4] the test was usually repeated on another sample of 10 insects if 2 or more individuals died in the first sample.

The criteria of effect of the added compounds or mixtures were: (1) Net mean mortality obtained from the formula $M=(x-y)100/x$, in which x represents the mean percentage of insects alive 72 hours

[4] The actual mortality for the control insects was 12 percent. In 312 insects fed fresh corn foliage during the experimental period, a smaller proportion, 5.4 percent, died of natural causes. Normal mortality was, therefore, moderate in the populations from which the test insects were drawn.

after feeding ad libitum on the bran-water standard, and y the mean percentage alive after feeding on the experimental bait; (2) mean survival time, the mean interval in hours from cessation of feeding until death, death being the failure of the insect to respond by movement to pinching of the tarsi or palpi with forceps; (3) mean bait consumption, the mean quantity in grams of bait eaten; (4) mean feeding time, the mean interval in minutes from beginning to cessation of feeding; and (5) milligram/gram, the mean quantity of compound or mixture in the consumed bait calculated as milligrams per gram of body weight of the insect.

THEORETICAL COMBINATIONS OF MORTALITY, BAIT CONSUMPTION, AND FEEDING TIME

In a series of data based on mortality with respect to a nontoxic food substance, there are only two combinations involving mortality, namely, a series without mortality and a series in which mortality is present. On the other hand, data on bait consumption from insects fed ad libitum may be grouped into three combinations expressing levels of bait intake with respect to the standard; that is, consumption higher, the same, and lower than that of the standard. Data on feeding time from insects fed in the same manner, likewise, may be placed in three groups; namely, time longer, the same, and shorter than that on the standard. There are then 2 possible mortality series and 9 possible combinations within each series, or a total of 18 theoretical combinations of mortality, bait consumption, and feeding time.

In the tabular arrangement of these theoretical combinations, given below, *M* indicates mortality, *C* bait consumption, and *T* feeding time, whereas the levels are indicated by *h*, *s*, and *l*, signifying, respectively, greater than, the same or similar to, and less than the standard.

Mh, Mortality greater than that of the standard food substance:
- *ChTh*, Very attractive toxic baits; eaten slowly.
- *ChTs*, Very attractive toxic baits; eaten in normal time.
- *ChTl*, Very attractive toxic baits; eaten voraciously.
- *CsTh*, Not markedly distasteful toxic baits; eaten slowly.
- *CsTs*, Toxic baits; consumption and feeding time like standard.
- *CsTl*, Toxic baits; consumption normal, but eaten rapidly.
- *ClTh*, Repellent toxic baits; eaten slowly.
- *ClTs*, Toxic baits; consumption small, but eaten in normal time.
- *ClTl*, Toxic baits; consumption small, but eaten in a short time.

Ms, Mortality not greater than that of the standard food substance:
- *ChTh*, Very attractive baits; eaten slowly.
- *ChTs*, Very attractive baits; eaten in normal time.
- *ChTl*, Very attractive baits; eaten voraciously.
- *CsTh*, Attractive baits; consumption normal but eaten slowly.
- *CsTs*, Attractive baits; consumption and feeding time like standard.
- *CsTl*, Attractive baits; consumption normal, but eaten rapidly.
- *ClTh*, Repellent nontoxic baits; consumption small, but eaten slowly.
- *ClTs*, Repellent nontoxic baits; consumption small, but eaten in normal time.
- *ClTl*, Repellent nontoxic baits; consumption small, but eaten in a short time.

RESULTS

The results are arranged in table 1 according to the combinations just discussed. Compounds which gave net mean mortalities of less than 15 percent are considered doubtfully toxic at the concentration employed.

TABLE 1.—*Effect on grasshoppers of compounds and mixtures in bran baits*

Compound or mixture in bait	Theoretical combination [1]	Mean bait consumption [2]	Mean feeding time [2]	Milligram/gram [3]	Mean survival time	Net mean mortality
		Grams	*Minutes*		*Hours*	*Percent*
Bran-water standard (no added chemical substance).		0.038	17			100
Zinc phosphide (98.7 percent Zn_3P_2)	*MhChTh*	**.055	**34	1.864	22	77
Thallous sulphate	*MhCsTh*	.046	**25	1.175	36	100
Ammonium fluoride	*MhCsTs*	.030	13	1.102	13	100
Potassium fluoride ($KF.2H_2O$)	do	*.049	15	1.641	13	100
Arsenic trioxide	do	.030	19	.822	37	77
Arsanilic acid	do	.039	20	1.082	42	15
Mercurous chloride	do	.030	18	.891	47	20
Crystal violet (dye content 94 percent)	do	.033	20	1.061	42	20
Diphenyl	do	.038	20	1.012	31	100
Ammonium bifluoride ($NH_4F.HF$)	*MhCsTl*	.028	**10	1.013	15	100
Zinc arsenite (tech.)	*MhClTs*	**.022	14	.715	47	93
Sodium arsenite (NaH_2AsO_3)	*MhClTl*	**.014	**8	.447	31	0
Magnesium carbonate	*MsChTh*	**.060	**38	2.270		0
Sodium benzoate	do	**.052	**38	1.890		0
Zinc cyanamide (tech.)	do	**.059	**34	1.874		0
Barium fluoride	*MsChTs*	**.057	19	1.928		0
Calcium fluoride	do	**.057	*22	1.745		0
Chromic oxide	do	**.052	17	1.619		0
Antimony pentoxide	*MsCsTh*	.037	**34	1.201		0
Antimony potassium tartrate (U. S. P.)	do	.046	**24	1.230		0
Bismuth trioxide	do	.041	**25	1.152		0
Lead benzoate	do	.044	**31	1.554		0
Lead chromate	do	.038	**24	1.104		3
Magnesium acetate [$Mg(C_2H_3O_2)_2.xH_2O$]	do	*.048	**24	1.566		0
Potassium permanganate	do	*.052	**24	1.562		0
Stannous chloride ($SnCl_2.2H_2O$)	do	.038	**23	.976		3
Acetone sodium bisulphite	do	.036	**31	1.101		0
Hexachlorobenzene	do	.034	**25	.930		0
Triphenylmethane	do	*.048	**30	1.227		0
Triphenylguanidine	do	.044	**30	1.219		0
Ammonium oxalate	*MsCsTs*	.029	20	.815		0
Antimony trioxide	do	.045	16	1.043		0
Barium chromate	do	.038	21	1.139		0
Bismuth oxychloride	do	.033	16	.967		0
Bismuth subcarbonate	do	.044	21	1.137		0
Bismuth subnitrate	do	.039	20	1.164		0
Boric acid	do	.037	*22	1.246		0
Cadmium chloride	do	.034	16	1.054		3
Cadmium nitrate [$Cd(NO_3)_2.4H_2O$]	do	.039	19	1.149		0
Cadmium oxide	do	.038	20	1.099		0
Cadmium sulphate ($3CdSO_4.8H_2O$)	do	.033	16	.992		0
Calcium carbonate	do	.043	16	1.179		0
Lead borate [$Pb(BO_2)_2.H_2O$]	do	.042	*25	1.244		0
Magnesium chloride ($MgCl_2.6H_2O$)	do	.032	16	1.311		0
Magnesium citrate	do	*.049	21	1.564		0
Magnesium fluoride	do	.041	*23	1.075		0
Magnesium oxide	do	.035	*23	.954		0
Magnesium oxalate ($MgC_2O_4.2H_2O$)	do	.042	20	1.113		0
Magnesium phosphate [$Mg_3(PO_4)_2.4H_2O$]	do	.032	22	.791		0
Magnesium sulphate, 12 percent (U. S. P.) ($MgSO_4.7H_2O$)	do	.028	15	11.154		0
Manganous sulphate ($MnSO_4.2H_2O$)	do	.039	19	1.099		0
Potassium bromate	do	.040	18	1.155		9
Potassium chrome alum	do	.039	20	1.092		0
Potassium ferricyanide	do	.034	22	1.078		0
Potassium ferrocyanide [$K_4Fe(CN)_6.3H_2O$]	do	*.050	20	1.423		0
Potassium iodide	do	047	21	1.230		0
Potassium methyl sulphate	do	*.053	22	1.544		0
Sodium iodate	do	.028	*11	.652		0
Sodium lauryl sulphate	do	.028	21	.785		0
Sodium sulphide ($Na_2S.9H_2O$)	do	.030	14	.931		9
Sulphur	do	.044	16	1.207		0
Crotonic acid	do	.033	20	1.173		0
Trithiomethylene	do	*.051	17	1.860		0
Copper cyanamide (tech.)	do	.035	25	1.034		9
p-Aminophenol	do	.039	17	1.097		0
o-Cyclohexylphenol	do	.037	21	1.136		0
p-Cyclohexylphenol	do	.046	*22	1.440		0
Sodium phenolsulphonate (U. S. P.)	do	.032	*11	.848		0
Tri-*o*-cresylphosphate	do	.037	14	.979		3
Resorcinol (U. S. P.)	do	.037	21	1.103		0
α-Naphthol	do	.027	22	.734		0

See footnotes at end of table.

TABLE 1.—*Effect on grasshoppers of compounds and mixtures in bran baits*—Continued

Compound or mixture in bait	Theoretical combination	Mean bait consumption	Mean feeding time	Milligram/gram	Mean survival time	Net mean mortality
		Grams	*Minutes*		*Hours*	*Percent*
β-Naphthol	*MsCsTs*	0.029	19	0.849		0
XXX Phenols [4]	do	*.028	*23	.817		0
p-Nitrodiphenyl	do	.031	22	.883		9
p-Phenetidine	do	.026	*11	.771		0
m-Dinitrobenzene	do	*.024	13	.579		0
Safranine bluish	do	.029	21	.722		0
Settled wood tar [4]	do	.030	16	.751		0
Sulphanilic acid	do	.044	18	1.085		0
Thiocarbanilide	do	.043	20	1.239		0
m-Phenylenediamine	do	.040	*23	1.134		0
Diphenylguanidine	do	.031	17	.920		0
α-Naphthylamine	do	.029	16	.735		0
β-Naphthylamine	do	.042	15	1.527		0
Diphenylamine sulphate	do	.036	16	1.184		0
Piperidinium tetrathiocyanatodiammino chromium.	do	.046	*21	1.222		0
Magnesium sulphate, 1 percent (U. S. P.)	*MsCsTl*	.034	**10	.972		0
Paraformaldehyde	do	.030	**10	1.006		0
Quinoidine [5]	*MsClTh*	**.023	**27	.718		0
Barium thiosulphate ($BaS_2O_3.H_2O$)	*MsClTs*	**.024	20	.732		0
Cupric borate	do	**.022	12	.618		0
Cupric carbonate	do	**.021	*11	.532		0
Cupric sulphate ($CuSO_4.5H_2O$)	do	**.022	11	.618		0
Sodium thiocyanate	do	**.023	16	.618		0
Phenol	do	**.012	14	.363		0
Tetrachlorophenol (tech.)	do	**.016	15	.385		0
Pentachlorophenol (tech.)	do	**.024	15	.689		0
Benzamide	do	**.015	21	.516		0
Acridine	do	**.023	13	.712		0
p-Nitrophenetole	do	**.022	19	.715		9
Nicotine peat [6]	do	**.016	18	.444		0
Cupric acetate [$Cu(C_2H_3O_2)_2.H_2O$]	*MsClTl*	**.013	**8	.400		0
Mercuric chloride	do	**.010	**6	.295		0
Magnesium sulphate, 35 percent (U. S. P.)	do	**.009	**5	7.666		0
3,5-Dinitro-o-cresol	do	**.011	**4	.299		3
Magnesium 2,4-dinitro-6-cyclohexylphenate	do	**.008	**5	.214		0
Acetophenone	do	**.009	**4	.260		0
XXX Oil [4]	do	**.022	**9	.568		0
p-Dichlorobenzene	do	**.016	**7	.452		3
Naphthalene	do	**.022	**8	.609		0
o-Nitroaniline	do	**.008	2	.247		0
Isoquinoline	do	**.009	**8	.312		3

[1] *M* signifies net mean mortality, *C* bait consumption, *T* feeding time; and with respect to a nontoxic food substance, *h* indicates greater than, *s* the same as or similar to, and *l* less than.

[2] ** Signifies highly significant ($P=0.01$); * signifies a significant difference ($P=0.05$).

[3] Milligrams of added chemical per gram of body weight of the insect.

[4] From destructive distillation of wood.

[5] Mixture of amorphous alkaloids from cinchona bark.

[6] Peat containing 10 percent of total nicotine; 90 percent of the nicotine is insoluble in water when the aqueous mixture contains about 0.5 percent of nicotine peat.

Survival times are approximate. In some instances they could be determined closely; in others, particularly when the insect died during the night, the survival time was estimated. The data are valuable, however, when large differences are concerned.

The mean bait consumption for each experimental bait was tested by the *t* test for a significant deviation from mean consumption of the bran-water standard. Since the samples were small, only means which gave values for *t* corresponding with probabilities of 0.01 or less are considered significant. These are indicated in the table by two asterisks. Means that yielded *t* values corresponding with probabilities of 0.05 or less, but greater than 0.01, although usually regarded as significant in biological experiments, are not so considered in the interpretation of these results. They are marked by an asterisk

in the table. All values for bait consumption not so marked, unless qualified in the discussion following, are interpreted as probably equivalent or similar to mean consumption of the bran-water standard.

Feeding time is more variable, and its precise determination therefore more difficult, than that of bait consumption. Feeding time is subject to several errors. To avoid disturbance, the feeding insect must not be observed too closely. During long feeding intervals the insect may stop eating for a time, although from its position before the bait container it may appear to be feeding actively. Whenever such pauses were noted they were timed, and the time was deducted from the total feeding time. But it was not possible to detect them all; consequently, long feeding times are subject to greater error than short times. On cool days, the insects fed more slowly than on warm days. During cool weather, experimentation was usually discontinued, or the data were marked for later qualification. Again, certain compounds may have so affected the texture or other physical properties of the bait that the rate of feeding was reduced. Notwithstanding these inaccuracies, feeding time has been of considerable value in the interpretation of the action of certain chemicals. The data were tested for significant deviations from the bran-water standard in the manner described for bait consumption, and the means in table 1 are marked by asterisks in the same way.

DISCUSSION

The conclusions regarding the effects of the chemical substances on mortality, bait consumption, and feeding time in this discussion apply strictly to the concentration employed, namely, 1 percent by weight of the moist bait. The only exception was magnesium sulphate which was tested at 1-, 12-, and 35-percent concentrations.

Lethal group.—Only 12 of the 110 compounds and mixtures tested were significantly lethal. The lethal compounds are assigned to six of the nine possible combinations of bait consumption and feeding time.

MhChTh.—Only one compound, zinc phosphide, can be placed in this group. Apparently *Melanoplus m. mexicanus* resembles *M. differentialis* (Thos.) in survival time and susceptibility to this compound (*10*). In comparison with sodium arsenite on *mexicanus*, survival time is shorter, but the quantity of bait eaten and the feeding time are much greater; from arsenic trioxide it differs in the significantly shorter survival time and the larger dose (milligram/gram). The higher bait consumption and longer feeding time compared with those for the standard bait are believed of actual significance.

MhCsTh.—A single compound, thallous sulphate, is placed in this group. Although the survival time is similar, its toxicity is lower than that for sodium arsenite. The long feeding interval may be the result of overtiming; however, the presence of this poison does not significantly affect the quantity of bait consumed.

MhCsTs.—Seven of the twelve toxic compounds are assigned to this category. Ammonium fluoride and potassium fluoride have the shortest mean survival times (13 hours) of any of the toxic compounds. The survival time for arsenic trioxide is about equal to that for sodium arsenite, and probably equivalent to the survival times of

the other compounds in this category, except the two fluorides. Arsanilic acid ($p\text{-}H_2NC_6H_4AsO_3H_2$) is apparently less toxic than the other arsenic compounds used in this investigation. Brinley (*1*) tested a number of aromatic compounds of arsenic on the eastern tent caterpillar (*Malacosoma americana* (Fab.), none of which proved more toxic than the inorganic arsenates. Mercurous chloride was perhaps slightly toxic though certainly not repellent, whereas mercuric chloride (cf. *MsClTl*) was so highly repellent that little of the bait was eaten. Crystal violet, a triphenylmethane dye, which Campbell (*2*) reported highly toxic by mouth and by injection to silkworms (*Bombyx mori* L.), and diphenyl were just slightly toxic at the concentration used.

It is of interest that more than half the baits containing toxic compounds were eaten in quantities and in a time equal or similar to that of the bran-water standard.

MhCsTl.—Only ammonium bifluoride falls in this category. In toxicity, survival time and bait consumption, the data are not distinguishable from those for the two fluorides in the group above. But the feeding time is shorter. Additional information would possibly change the group locations of these fluorides.

MhClTs.—Zinc arsenite, the single compound in this category, resembled sodium arsenite in toxicity and bait consumption, yet the feeding time was longer.

MhClTl.—Sodium arsenite is the only compound which can be assigned to this category. On the basis of As_2O_3 concentration, it was slightly less toxic than As_2O_3 itself, but the difference is probably of no significance. The survival time was similar to that of the other arsenic compounds tested, except zinc arsenite, and significantly longer than the survival times of the soluble flourides. However, bait consumption was the smallest, feeding time the shortest of the toxic compounds examined. The results appear to support a former conclusion from a study of the effect of sodium arsenite on *Melanoplus femur-rubrum femur-rubrum* (DeG.) and *M. differentialis* that "the grasshoppers were not repelled by the odor or taste of the bait mixtures used, and ceased to feed only when they became satiated or were too sick to eat more" (*9, p. 1078*).

Nontoxic group.—Eight of the nine theoretical combinations were realized. The only combination to which a compound could not be assigned was MsChTl. Such a compound, so definitely attractive that large quantities of the bait containing it would be devoured in a short time, would seldom be found among substances selected for their toxic or repellent action.

MsChTh.—Three compounds are assigned to this category. The attractiveness of zinc cyanamide, a technical product, may have resulted from impurities. The apparent attractiveness of magnesium carbonate and sodium benzoate cannot, at present, be related to their physical or chemical properties.

MsChTs.—The three compounds in this category appeared to stimulate increased bait consumption. Barium and calcium flourides, relatively insoluble compounds, are apparently without toxicity to the insect used.

MsCsTh.—Twelve compounds, grouped here, showed significantly long feeding times. Although overtiming may have been frequent in this group, mean feeding times of 30 minutes or more probably have

actual significance. Some of these compounds may have rendered the bait somewhat distasteful, or have changed its physical properties with a resulting increase of feeding time; but direct observations are not available on these points. Lead chromate and stannous chloride were perhaps very slightly toxic at the concentrations used.

MsCsTs.—About half the nontoxic compounds fall in this category, in which there are no striking differences from the bran-water standard. A few compounds in this group gave net mortalities as high as 9 percent, and a few showed significant differences from the standard in bait consumption and feeding time that in larger samples might become highly significant. Noteworthy is the almost complete lack of toxicity of the cadmium compounds. Several investigators (*4, 5*) have found certain cadmium compounds toxic to a number of species of insects. The seven magnesium compounds proved to be without toxicity, a result anticipated from the conclusions of several workers which were published while this investigation was in progress (*3, 6, 11*). Like the barium and calcium fluorides, the insoluble magnesium fluoride is nontoxic. Diphenylguanidine, reported by Ginsburg and Granett (*5*), to be quite toxic and repellent to silkworms, was without effect on *Melanoplus m. mexicanus* at the lower concentration employed in these tests.

MsCsTl.—Two compounds are assigned to this category. Magnesium sulphate at 1-percent concentration gave a short feeding time, whereas at 12-percent, the feeding time was normal.

MsClTh.—Quinoidine, a mixture of amorphous alkaloids from cinchona bark, evidently rendered the bait so unattractive that it was nibbled over a long feeding period. It is possible the actual feeding time was shorter than that given. This substance is reported as somewhat toxic though not repellent to the silkworm (*5*).

MsClTs.—There are 12 compounds in this group. Bait consumption was significantly low, and the feeding times for most of the compounds were shorter than that for the standard, although the differences are not significant. Three of the five copper compounds examined appear in this group. Larrimer (*8*) found cupric sulphate the most repellent of a number of substances for several species of grasshoppers and crickets. Pentachlorophenol, reported as rather toxic though not repellent to the silkworm (*5*), proved rather repellent and not toxic to *Melanoplus m. mexicanus* at the concentration used in these tests.

MsClTl.—Eleven compounds, all highly repellent, fall in this group. Only 2 of them, mercuric chloride and magnesium sulphate, the latter fed at the enormous concentration of 35 percent, are inorganic compounds. The results from magnesium 2,4-dinitro-6-cyclohexylphenate are in accord with those obtained by Kagy (*7*) with the calcium salt of this compound on *Melanoplus f. femur-rubrum.* The calcium salt was so distasteful to the latter insect that attempts to feed a lethal dose were futile, whereas the phenol itself was more than twice as toxic as arsenic trioxide. Although isoquinoline proved somewhat toxic and strongly repellent to silkworms (*5*), it was at most very slightly toxic and strongly repellent to *M. m. mexicanus* at 1 percent.

The data for the toxic compounds were examined for sex differences. The 4 survivors of the 66 insects that ate the sodium arsenite bait were females; and the females consumed a larger absolute quantity

of this bait, and a larger quantity relative to the body weight than the males. The difference between the means of bait consumption for the sexes was significant, but not highly significant. The means for survival time and feeding times were not significantly different. Nine of the fourteen insects that survived the diphenyl bait were females, and the size of the dose in milligrams/gram consumed by the females was highly significant, in fact, nearly twice that eaten by the males. No other differences in the effect of the toxic compounds on the sexes were as clearly suggestive of a significant sex difference as these.

The insects fed the bran-water bait consumed, before they ceased to eat, 0.10 g of the bait per gram of body weight. No significant sex differences were found in the quantity consumed relative to body weight. On the basis of a mean bait consumption of 0.038 g., and a mean body weight for both sexes of 0.384 g., these grasshoppers consumed approximately 10 percent of their weight at one feeding. This figure is smaller than the value (16 percent) obtained for the consumption of a bran-molasses-water bait by *Melanoplus f. femur-rubrum* (*9*).

SUMMARY

One hundred and ten inorganic and organic substances were fed individually in bran-water baits to adult lesser migratory grasshoppers (*Melanoplus m. mexicanus* Saussure), the chemical substance constituting 1 percent by weight of the moist bait. The insects were allowed to feed ad libitum on the bait mixture. The net mean mortality within 72 hours, mean survival time in hours, mean bait consumption in grams, mean feeding time in minutes, and mean quantity of chemical substance consumed with the bait in milligrams per gram of body weight of the insect were recorded. The theoretical combinations of mortality, levels of bait consumption, and levels of feeding time with respect to a nontoxic food substance are tabulated and the results for each bait are grouped and discussed under the appropriate combination. Conclusions are limited strictly to the effects produced by the chemical substances at 1-percent concentrations.

The lethal compounds are grouped as follows: (1) Highly toxic compounds (93- to 100-percent mortality)—zinc phosphide, ammonium fluoride, ammonium bifluoride, potassium fluoride, arsenic trioxide, sodium arsenite, and zinc arsenite; (2) moderately toxic compounds (77-percent mortality)—arsanilic acid and thallous sulphate; (3) slightly toxic compounds (15- to 20-percent mortality)—crystal violet, diphenyl and mercurous chloride. The three fluorides gave the shortest survival times, zinc phosphide was intermediate with 22 hours, and the remaining lethal compounds ranged from 31 to 47 hours. Highest bait consumption in the lethal group was given by zinc phosphide, lowest by sodium arsenite and zinc arsenite. The insects fed longest on baits containing zinc phosphide, but only a brief period on the ammonium bifluoride and sodium arsenite baits. Sodium arsenite is outstanding in the small quantity of poison relative to body weight required for high toxicity.

The nontoxic substances varied greatly in their effect on bait consumption and feeding time. Consumption was greatest of baits containing magnesium carbonate, sodium benzoate, zinc cyanamide,

barium fluoride, and chromic oxide, and smallest of baits containing a rather large group of compounds among which were most of the copper compounds and many of the phenols used in these tests. Feeding time was abnormally long, or equal to that on the bran-water standard, on compounds that showed high bait consumption; but it was also very long on some chemicals that were consumed in normal quantities. Some of these long feeding times may have resulted from the effect of the compound on the texture or other physical property of the bait. All the cadmium and magnesium compounds were ineffective except magnesium sulphate, which was repellent at the enormous concentration of 35 percent. The insoluble fluorides of barium, calcium, and magnesium were nontoxic. Mercuric chloride was so repellent that little of the bait containing it was eaten.

Sodium arsenite and diphenyl are probably more toxic to the males than to the females.

If undisturbed, this grasshopper consumes about 0.10 g of a nontoxic bran-water bait per gram of body weight, or 10 percent of its weight at one feeding.

LITERATURE CITED

(1) Brinley, F. J.
1926. Insecticidal value of certain war chemicals as tested on the tent caterpillar. Jour. Agr. Research 33: 177–182.
(2) Campbell, F. L.
1932. Preliminary experiments on the toxicity of certain coal-tar dyes for the silkworm. Jour. Econ. Ent. 25: 905–917, illus.
(3) Frings, Hubert W., and Frings, Mable S.
1937. Magnesium sulfate—a new insecticide. Science 85: 428.
(4) Ginsburg, Joseph M.
1934. Toxicity of cadmium to chewing insects. Science 80: 269.
(5) ——— and Granett, Philip.
1935. Arsencial substitutes. I. Chemicals tested as arsenical substitutes in 1934. Jour. Econ. Ent. 28: 292–298.
(6) Howard, Neale F.
1937. Magnesium sulfate valueless as a control for the bean beetle. Science 86: 286–287.
(7) Kagy, J. Franklin.
1936. Toxicity of some nitro-phenols as stomach poisons for several species of insects. Jour. Econ. Ent. 29: 397–405.
(8) Larrimer, Walter H.
1921. Grasshopper and cricket repellents. Jour. Econ. Ent. 14: 259–263.
(9) Richardson, Charles H., and Haas, Louise E.
1932. The evaluation of stomach poisons for grasshopper baits. Jour. Econ. Ent. 25: 1078–1088, illus.
(10) ——— and Thurber, George A.
1933. Further studies on the relative toxicity of poisons for grasshopper baits. Jour. Econ. Ent. 26: 494–499.
(11) Smith, Roger C.
1937. Magnesium sulfate—an unsatisfactory substitute for arsenicals in grasshopper baits. Science 86: 226–228.

PROTEOLYTIC ACTIVITY IN RELATION TO THE BLACKENING OF POTATOES AFTER COOKING [1]

By A. FRANK ROSS, *research assistant in agricultural chemistry*, and W. E. TOTTINGHAM, *associate professor of agricultural chemistry, Wisconsin Agricultural Experiment Station*

INTRODUCTION

In a previous paper (*15*) [2] a general survey was made of the factors related to the development of gray to black discoloration in boiled potatoes (*Solanum tuberosum* L.). Evidence was there presented which indicated instability of the protein in abnormal tubers, and it was concluded that this condition is associated with the accumulation of tyrosine, tryptophane, and other amino acids. Other observers of such accumulation (*8*, *11*, *13*) noted its correlation with a deficiency of potassium in plant tissue. Thus the nutritional and enzymic aspects of plant existence become associated, and it is apparent that abnormal activity of enzymes may follow upon malnutrition. Hartt (*8*) concluded that the accumulation of amino acids, which she observed in potassium-deficient sugarcane, was the combined result of diminished synthesis and translocation of protein. The present paper covers an investigation of proteolytic activity as a causal factor in the blackening of cooked potatoes. Since the early paper of Vines (*16*) mentioning the presence of "tryptase" in the potato, little data have been reported with reference to the proteolytic enzymes of the potato tuber.

Since most of the protein of potato tubers is soluble in the sap (*2*) it appeared advisable to use sap as the substrate. The proteolytic activity was determined by following the rate of liberation of amino acids during the autolysis of tuber tissue. As tyrosine seemed to be a primary factor in discoloration, particular attention was given to the release of this constituent. Potato tubers rate high in tyrosinase activity and, consequently, that enzyme must be inhibited; otherwise, much tyrosine may be lost through oxidation. Most of the inhibitors of tyrosinase are known to activate certain plant proteases, so that their use might introduce complications. Hence it appeared advisable to allow autolysis to proceed under anaerobic conditions.

Another reason for the choice of this treatment arose from the work of Paech (*12*) on autolytic decomposition of proteins in green tissues. He found that the activity of protease was readily modified by atmospheric oxygen after cell destruction, and that this oxidation was preliminary to activation. Since oxygen tension within the tuber is quite low, exclusion of air would approach the natural condition of the tissue in this respect.

[1] Received for publication March 19, 1938; issued September 1938. This work was supported by grants from the Wisconsin Alumni Research Foundation, University of Wisconsin.

[2] Italic numbers in parentheses refer to Literature Cited, p. 440.

PROCEDURE

Representative samples of normal and of abnormal tubers were washed, wiped carefully, and frozen with solid carbon dioxide. While still thoroughly frozen the samples were ground in a chilled meat grinder. Portions of the well-mixed tissue were placed in cooled flasks and an equal weight of oxygen-free water was added. Toluene was added as a preservative. In some cases twice the above weight of water was used. The flasks were fitted with rubber stoppers through which passed inlet and outlet tubes. Nitrogen gas, free of oxygen, was then bubbled through for several minutes. After the flasks were sealed they were allowed to stand (with occasional shaking) at room temperature for several hours. They were then placed in an ice box overnight to obtain complete thawing and exosmosis of cell contents, with a minimum of enzymatic change. During the next morning the digests were allowed to come to room temperature and samples were taken. After this they were incubated at 37° C. and sampled at intervals.

While the samples were being taken nitrogen was bubbled through the suspensions at such a rate as to provide thorough stirring. Portions were removed by means of a 50-cc pipette (with tip enlarged) and immediately added to 1 cc of 50-percent trichloroacetic acid. The preparations were mixed, allowed to stand one-half hour and then centrifuged. This procedure usually resulted in a clear, slightly amber-colored solution. Aliquot parts were taken from this for analysis.

To lessen the possibility that other variables than proteolytic activity might be included in this experimental treatment, the tubers selected for each test were of practically the same size and of the same variety. Analyses had proved that samples so selected show little or no consistent differences in hydrogen-ion concentration, moisture, or total protein content, or in the amount of protein in the prepared digests. The average results from several different tests should, therefore, be dependable. As the hydrogen-ion concentration of the digests remained practically constant during digestion, it was unnecessary to employ buffers.

A difference in the rate of hydrolysis during autolysis might equally well be due to either inequality in the ease of cleavage of proteins or variations in enzymic activity. As a test of these alternatives the enzyme concentration was increased by the addition of papain. The equivalent of 0.2 g per 100 g of tissue was added from a suspension in water of a commercial preparation of the enzyme. Controls were prepared by substituting an equal volume of water for the enzyme suspension. In other digests the amount of protein was increased by the addition of either potato protein (from normal tubers) or gelatin, to the extent of 2 percent of the tissue.

In certain tests the concentration of potassium in digests of abnormal tubers was made equal to, or in excess of, that of normal tubers, by the addition of 0.2 g of potassium in the form of potassium chloride. In some experiments sodium chloride was added to the autolysates (7.5 g per 100 g of tissue) for the purpose of possibly increasing the concentration of soluble protein.

DETERMINATION OF TYROSINE AND OF TRYPTOPHANE

An aliquot portion of the digest (commonly 10 cc) was pipetted into a 100-cc volumetric flask and diluted with 30 cc of water. Twenty-five cubic centimeters of a saturated solution of sodium carbonate was then added, followed immediately by 5 cc of Folin and Ciocalteu's (*3*) phenol reagent. After standing for one-half hour, the contents of the flasks were diluted to volume, and the color was compared with that developed by a tyrosine standard similarly prepared.

In tests not reported here it was found that this procedure gives a much higher result than can be accounted for by the sum of tyrosine and tryptophane when determined separately by the procedure cited. It was considered likely here that any changes occurring in this fraction would be due primarily to shifts in the amount of these amino acids. However, because of the possibility of other changes, the content of α-amino nitrogen in digests was determined also.

DETERMINATION OF α-AMINO NITROGEN

The method employed for determining α-amino nitrogen was a modification of the Sorensen formaldehyde titration. It was found that the comparator technique of Grünhut (*7*) and of Lüers (*9*) could be satisfactorily applied. The concentration of indicators and the volume of solutions at the time of comparison were carefully controlled. Titration was accomplished in two steps: (1) In aqueous solution to an end point corresponding to pH 8.5 (using phenolphthalein); and (2) from this end point to the same color after the addition of neutral formaldehyde. Titer 2 is a measure of the liberated amino nitrogen. It was found that the use of N/70 sodium hydroxide gave duplicate determinations of good agreement.

The preparation of neutral formaldehyde deserves special comment. It was observed that if 40-percent formaldehyde, adjusted to a faint pink with phenolphthalein, was diluted several times with water, a very noticeable increase in the indicator color resulted. When the neutralized formaldehyde was added to a sample containing a small amount of α-amino nitrogen (step 2) there resulted not the expected discharge of the pink color, but its intensification. This effect was noticed also by Mounfield (*10*), who eliminated the error through individual correction in each case by adding the sodium hydroxide equivalent of the apparent development of alkalinity. In the present investigation this error was eliminated by adjusting the formaldehyde to such a pH value as to give the desired shade of indicator upon dilution in the titration. Precautions were taken to insure the proper concentration of indicator.

RESULTS AND DISCUSSION

The results of the first several preliminary experiments were somewhat erratic but indicated, on the whole, a significant difference in the proteolytic activity of discoloring and normal tubers. Proteolytic activity was generally greater in the digests of discoloring tubers than in that of normal tubers. The average results of three of the more reliable tests are shown in figure 1, disclosing a substantially

greater activity in the abnormal tubers. Subsequent experiments were conducted over a longer period and the average results of three tests are shown in figure 2. These data show greater activity than preceding tests over the 6-day period because some of the earlier tests gave unusually low proteolytic activity. Differences in variety, age, size, and other qualifications of the tuber may account for the occasional variation of activity noted in different experiments. It was

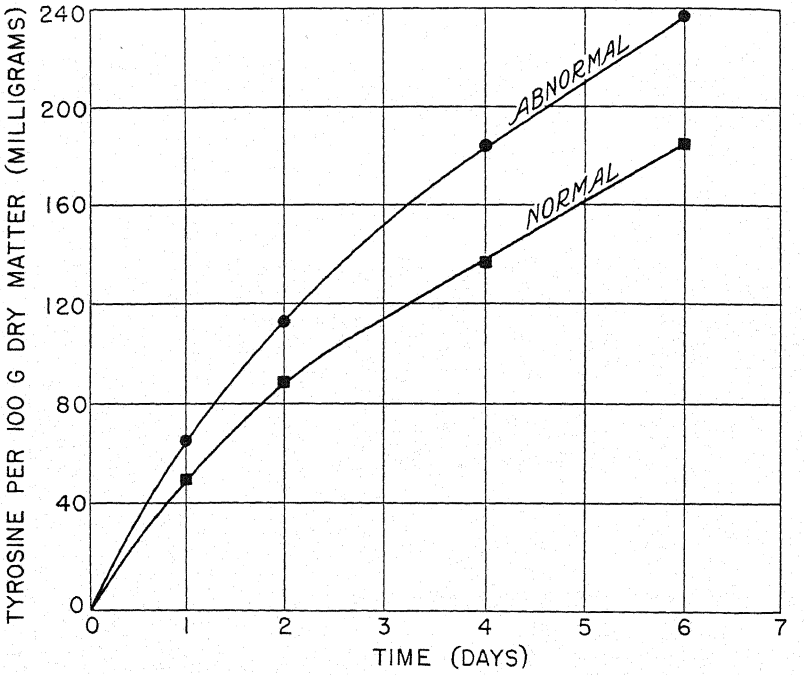


FIGURE 1.—Liberation of tyrosine during the autolysis of normal and of abnormal potato tissue in the first set of experiments.

found that the larger addition of water, 2 cc per gram of fresh tissue, entailed a slightly greater hydrolysis per gram of dry matter.

The data for α-amino nitrogen, as presented in figure 3, also show a significant increase of proteolysis in the discoloring tubers. However, the differences are not as great as in the case of the tyrosine equivalent. This relation is in agreement with the earlier evidence that tyrosine accumulates more than other amino acids in discoloring tubers and seems to be one of the first components released from protein (*1*, *5*). In spite of this difference in amino acid content of the tissue the isolated proteins appear to be identical in respect to the content of nitrogen, tyrosine, and tryptophane. The proteins were extracted with 5-percent solution of sodium chloride, reprecipitated several times with magnesium sulphate, and finally precipitated by alcohol. Tyrosine and tryptophane were determined by the method of Folin and Marenzi (*4*). Protein from normal potatoes contained 13.95 percent nitrogen, 5.37 percent tyrosine, and 0.52 percent tryptophane, while that from abnormal potatoes contained 13.90, 5.38, and 0.52 percent of the respective constituents. This content of tyrosine is

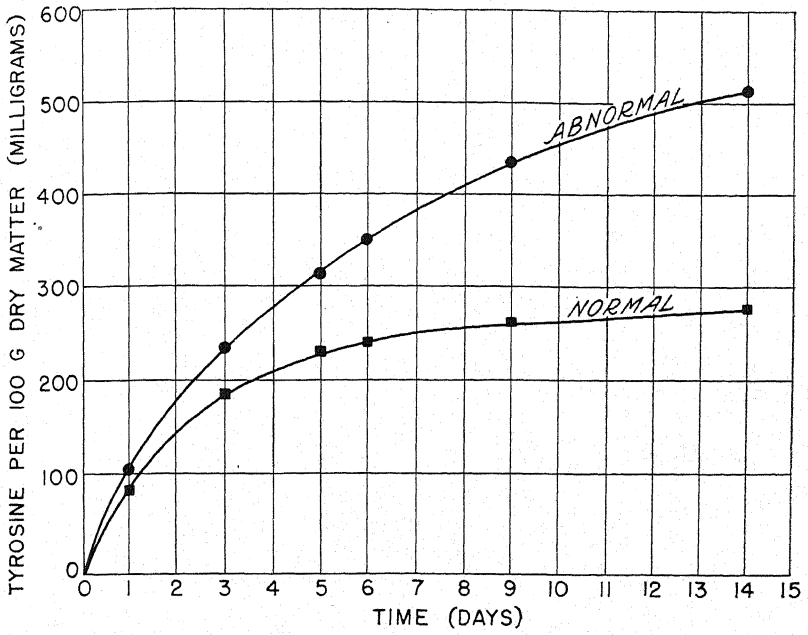


FIGURE 2.—Liberation of tyrosine during the autolysis of normal and of abnormal tissue in the second set of experiments.

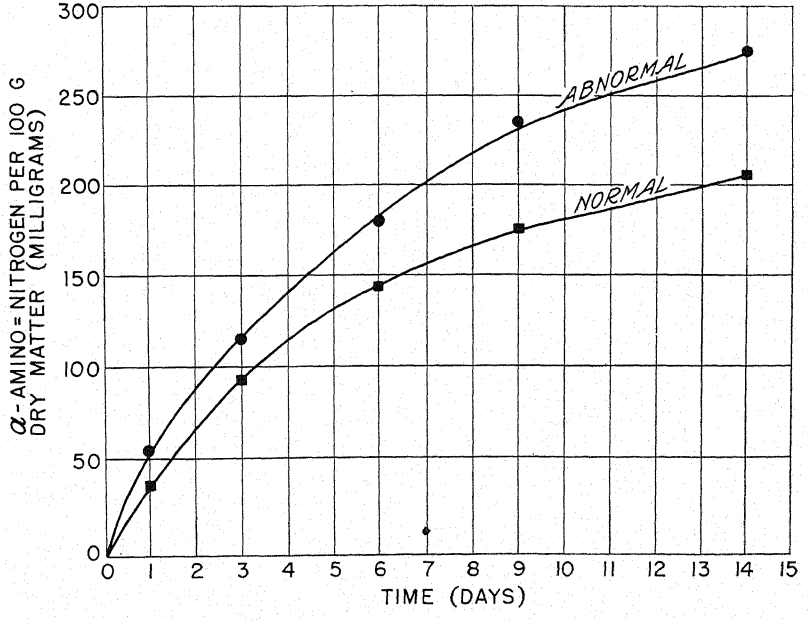


FIGURE 3.—Liberation of α-amino nitrogen during the autolysis of normal and of abnormal potato tissue.

higher than any previously reported for tuberin. Sjollema and Rinkes (*14*) reported 4.3 percent of this constituent. No report of the tryptophane content of tuberin has been found in the literature. Fürth and Lieben (*6*) applied alkaline hydrolysis to the tuber tissue and reported 0.2 percent tryptophane in the dry matter which they computed as equivalent to 3.3 percent in the protein. The constancy of tyrosine and tryptophane content in these protein preparations suggests that either this molecular complex is stabilized by precipitation in the abnormal tuber extracts or that polypeptides are involved in the greater susceptibility of such tubers to proteolysis. In the

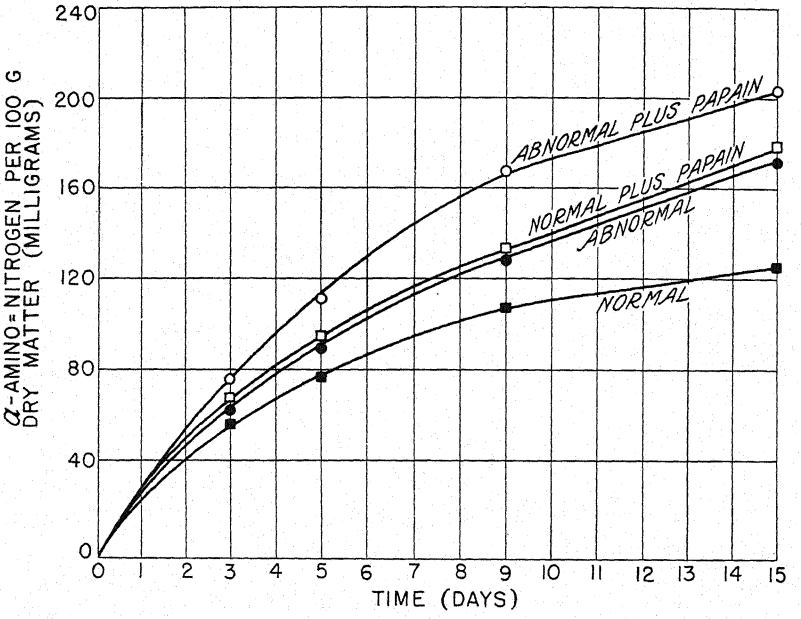


FIGURE 4.—Liberation of α-amino nitrogen in autolysates alone and in autolysates plus papain.

writers' earlier work (*15, p. 302*) the isolated proteins differed in ease of alkaline hydrolysis.

When the enzyme concentration was increased by the addition of papain an acceleration in the rate of hydrolysis resulted in each type of digest. The results of a single experiment are given in figure 4, but these data were substantiated by two tests which gave similar, though more irregular, curves. While the difference is generally rather small, the departures up to the twelfth day show somewhat greater acceleration by papain in the digests of abnormal tuber tissue. If there were inequality in enzyme activity of the two types of tissue, the addition of equal proportions of another enzyme should result in the suppression of differences in rates of autolysis. Relatively severe depletion of the substrate may have prevented more marked acceleration of proteolysis in abnormal tissue following the addition of enzyme. The results thus seem to be indicative of the presence of either easily hydrolyzed protein or lower polypeptides in discoloring pota-

toes. They do not preclude the possibility of difference in activity of the native enzymes.

A further matter of interest here is the relative positions at which the curves flatten. In the case of normal tubers, the curves of autolysis begin to level off about the seventh or eighth day (fig. 4). No such decline in rate of proteolysis was apparent in either the autolysates of abnormal tissue or the digests of normal tissue with papain. On the other hand, the curve for digest of abnormal tissue with papain (fig. 4) begins to flatten about the eleventh or twelfth day. Normally, one would expect the decline in rate of hydrolysis to occur first in the system in which hydrolysis is most rapid (abnormal tuber digests), as a more serious depletion of substrate would occur. It may be suggested that the more readily hydrolyzed substrate of discoloring potatoes could include polypeptides, a protein of low molecular weight, or an unstable form of a larger protein structure. When protein was added to the digests, proteolysis still proceeded at a somewhat greater rate in tissue from discoloring tubers, but the spread in activity was lessened. This effect offers limited evidence that the enzymes of the two tuber types are equally active on the added protein. In general, the results support earlier evidence (*15, p. 302*) of instability of the protein, as well as the concept of lower polypeptides, in the abnormal tubers.

It proved infeasible to add cyanide for the activation of protease because this ion interfered with the determination of tyrosine and tryptophane. The addition of sodium chloride for its possible solvent effect upon protein did not affect the rate of hydrolysis appreciably. An increase in the potassium concentration of digests of discoloring potatoes, making this factor essentially equal to that of normal ones by the addition of potassium chloride, did not decrease the rate of hydrolysis. The possibility is recognized that other sources of this metal might influence the rate of hydrolysis.

Consideration of the data for α-amino nitrogen shows that the differences were significant and not merely magnified by the method of expression as milligrams per 100 g of dry matter. Expressed as cubic centimeters of N/70 sodium hydroxide, the titration of 5 cc normal digest increased from 0.6 after 1 day to 3.6 after 14 days, while the discoloring digests showed increases over these levels of 50 and 36 percent, respectively. The addition of the doubled proportion of water (2 cc per gram) increased the release of amino nitrogen by about 50 percent but left the spread between samples undisturbed. Duplicate titrations generally agreed within 0.1 cc.

The liberation of more than 200 mg of α-amino nitrogen per 100 g of dry matter, as observed in the present work, represents a considerable hydrolysis of protein. This is evident from the fact that there is about 5 percent of true protein in the dry matter of potato tubers, which is equivalent to about 800 mg of total nitrogen in 100 g of dry matter.

During the course of this investigation, data were accumulated on the content of α-amino nitrogen and of tyrosine equivalent in the tuber extract as prepared. By tyrosine equivalent is meant the entire reacting capacity of the extract obtained, when sodium carbonate is added before the phenol reagent in the usual manner. Calculated on the basis of the total dry matter, normal tubers contained 0.35 percent of α-amino nitrogen and 0.43 percent of tyrosine equivalent. Discol-

oring tubers were 28.6 percent and 39.5 percent higher in content of these respective constituents. These differences are much greater than those reported previously (*15, p. 299*), but in the earlier examination the tubers had not been indexed for discoloration after cooking. Furthermore, a different type of extract was used here, namely, sap acidulated with trichloroacetic acid, whereas in the earlier work alcohol to give about 60 percent concentration was employed.

SUMMARY

Reasons are given for considering abnormal enzymic activity as a possible cause for the blackening of potatoes after cooking.

Procedures are described for the investigation of autolysis in potato tuber tissue, and modified methods for the determination of α-amino nitrogen and of a tyrosine equivalent are given.

Discoloring tubers show a considerable increase of the tyrosine equivalent during autolysis as compared with normal tubers. The latter release over 25 percent of their protein or peptide nitrogen as α-amino nitrogen under favorable conditions for autolysis, but there is an appreciable increase of this function in discoloring tubers.

Proteins prepared by salting out from the saps of normal and discoloring potatoes contained equal amounts of both tyrosine and tryptophane. The amounts of these constituents differ from values previously reported.

Earlier evidence of the presence of abnormal proportions of free amino acids and tyrosine in discoloring potatoes is confirmed. By using indexed tubers, an increase of nearly 30 percent in amino nitrogen and about 40 percent in tyrosine equivalent was found in discoloring potatoes as compared with normal ones.

Sodium chloride, used as a solvent for tuberin, did not increase the rate of proteolysis. Potassium chloride did not suppress the rate of proteolysis, thus indicating that a deficit of this form of potassium was not the direct cause of accumulation of amino acids.

Added proteins were hydrolyzed at essentially equal rates in tissue digests of the two types of tuber. From this it appears that increase of free amino nitrogen in discoloring tubers is not due primarily to abnormal activity of proteolytic enzymes.

Papain increased proteolysis somewhat more in the digest of discoloring potatoes. These results are interpreted as indicative of either decreased stability of proteins or increased proportions of lower polypeptides as causal factors in the increased free amino acid content of the abnormal tubers.

LITERATURE CITED

(1) Abderhalden, Emil, and Reinbold, Béla.
1905. die monoaminosäuren des "edestins" aus sonnenblumensamen und dessen verhalten gegen pankreassaft. Hoppe-Seyler's Ztschr. Physiol. Chem. 44: [284]–293.

(2) Cohn, Edwin J., Gross, Joseph, and Johnson, Omer, C.
1919. the isoelectric points of the proteins in certain vegetable juices. Jour. Gen. Physiol. 2: 145–160, illus.

(3) FOLIN, OTTO, and CIOCALTEU, VINTILA.
1927. ON TYROSINE AND TRYPTOPHANE DETERMINATIONS IN PROTEINS. Jour. Biol. Chem. 73: 627–650.
(4) ——— and MARENZI, A. D.
1929. TYROSINE AND TRYPTOPHANE DETERMINATIONS IN ONE-TENTH GRAM OF PROTEIN. Jour. Biol. Chem. 83: 89–102.
(5) FÜRTH, OTTO, and FISCHER, ANTON.
1924. ÜBER DIE ERMITTLUNG DES TYROSINGEHALTS VON PROTEINEN. III. Biochem. Ztschr. 154: 1–23.
(6) ——— and LIEBEN, FRITZ.
1921. COLORIMETRISCHE UNTERSUCHUNGEN ÜBER DAS TRYPTOPHAN. VI. ÜBER DEN TRYPTOPHANGENHALT EINGER NAHRUNGSMITTEL UND DEN TRYPTOPHANBEDARF DES ERWACHSENEN MENSCHEN. Biochem. Ztschr. 122: 58–85.
(7) GRÜNHUT, L.
1919. DIE BESTIMMUNG DES AMINOSÄUREN-STICKSTOFFS, INSBESONDERE IN SUPPENWÜRZEN UND ERSATZBRÜHWÜRFELN. Ztschr. Untersuch. Nahr. u. Genussmtl. 37: 304–324, illus.
(8) HARTT, CONSTANCE ENDICOTT.
1934. SOME EFFECTS OF POTASSIUM UPON THE AMOUNTS OF PROTEIN AND AMINO FORMS OF NITROGEN, SUGARS, AND ENZYME ACTIVITY OF SUGAR CANE. Plant Physiol. 9: 453–490, illus.
(9) LÜERS, HEINRICH.
1920. STUDIEN ÜBER DIE REIFUNG DER CEREALIEN. Biochem. Ztschr. 104: [30]–81.
(10) MOUNFIELD, JAMES DENIS.
1936. THE PROTEOLYTIC ENZYMES OF SPROUTED WHEAT. II. Biochem. Jour. 30: 1778–1786, illus.
(11) NIGHTINGALE, G. T., SCHERMERHORN, L. G., and ROBBINS, W. R.
1930. SOME EFFECTS OF POTASSIUM DEFICIENCY ON THE HISTOLOGICAL STRUCTURE AND NITROGEN AND CARBOHYDRATE CONSTITUENTS OF PLANTS. N. J. Agr. Expt. Sta. Bull. 499, 36 pp., illus.
(12) PAECH, KARL.
1935. ÜBER DIE REGULATION DES EIWEISSUMSATZES UND ÜBER DEN ZUSTAND DER PROTEOLYTISCHEN FERMENTE IN DEN PFLANZEN. Planta 24: [78]–129, illus.
(13) RICHARDS, F. J., and TEMPLEMAN, W. G.
1936. PHYSIOLOGICAL STUDIES IN PLANT NUTRITION. IV. NITROGEN METABOLISM IN RELATION TO NUTRIENT DEFICIENCY AND AGE IN LEAVES OF BARLEY. Ann. Bot. [London] 50: [367]–402.
(14) SJOLLEMA, B., and RINKES, I. J.
1912. DIE HYDROLYSE DES KARTOFFELEIWEISSES. Hoppe-Seyler's Ztschr. Physiol. Chem. 76: [369]–384.
(15) TOTTINGHAM, W. E., NAGY, RUDOLPH and ROSS, A. FRANK.
1936. THE PROBLEM OF CAUSES OF BLACKENING IN COOKED POTATOES. Amer. Potato Jour. 13: [297]–309, illus.
(16) VINES, S. H.
1909. THE PROTEASES OF PLANTS (VI). Ann. Bot. [London] 23: 1–18.

MORPHOLOGY OF THE DIGESTIVE TRACT OF THE BLACKFLY (SIMULIUM NIGROPARVUM)[1]

By JAMES A. COX

Assistant entomologist, Virginia Agricultural Experiment Station

INTRODUCTION

Comparatively little information exists in the literature relative to the internal anatomy of the family Simuliidae. Hungerford (*6*)[2] has figured and described the internal anatomy of *Simulium vittatum* Zett., and Smart (*11*) in a recent paper has described the internal anatomy of *S. ornatum* Mg.

In recent years it has been found that certain species of blackflies transmit disease-producing organisms. Blacklock (*1, 2*) has demonstrated that *simulium damnosum* Theob. is an intermediate host of a filarial worm *Onchocerca volvulus* Leuckart, which causes a disease of man. O'Roke (*10*) has shown that a blood protozoon of ducks, *Leucocytozoon anatis* Wickware, passes the sexual stage of its life cycle in *Simulium venustum* Say and that the organism is transmitted by the bite of the fly.

During the past 2 years the blackfly *Simulium nigroparvum* Twinn has been found feeding on turkeys in southwestern Virginia, and Johnson and Underhill (*8*) have shown that this fly is responsible for the transmission of a blood protozoon disease of turkeys. In view of the recent discovery that *S. nigroparvum* is an important vector of this blood protozoon of turkeys, the writer felt that a detailed study of the internal anatomy of the fly would be of service in studying the life history of the protozoon. Specimens of *S. nigroparvum* were readily accessible, and a detailed study was made of the internal morphology of this fly. Since only the females feed on turkeys and the males are unimportant as possible vectors, the digestive tract of the female only is described.

TECHNIQUE

Dissections were made on freshly killed specimens. For sectioning, the material was fixed in Bouin's fluid. All material was dehydrated with ethyl alcohol and cleared in terpineol. Hemalum and Delafield's haematoxylin were used for nuclear stains, and fast green and eosin for counter stains.

GROSS ANATOMY

The fore intestine (stomodaeum) is represented by the oesophagus and its diverticulum, the crop with its duct, and part of the cardiac valve. The mid intestine (mesenteron or ventriculus) is a simple tube, the anterior portion of which is narrow and the posterior portion

[1] Received for publication January 8, 1938; issued September 1938.

[2] Italic numbers in parentheses refer to Literature Cited, p. 447.

of which is expanded and is capable of considerable distention. The hind intestine (proctodaeum) comprises the distal intestine, the Malpighian tubules, the rectal pouch and glands, and the rectum proper.

MOUTH PARTS AND SUCKING APPARATUS

The mouth parts of several species of blood-sucking Nematocera have been figured and described by several workers, among whom may be mentioned Dimmock (*4*), Kellogg (*9*), Emery (*5*), Cameron (*3*), Jobling (*7*), and Smart (*11*). The mouth parts of *Simulium nigroparvum* are the piercing, sucking type and are quite similar to those described by Cameron (*3*) for *S. simile* Mal. and by Smart (*11*) for *S. ornatum*. Observations made on living females of *S. nigroparvum* have demonstrated that the mode of biting is quite similar to that of *Culicoides pulicaris* L. as described by Jobling (*7*).

The mouth parts and biting apparatus are shown in plate 1. In general character the mouth parts are short and broad. The labrum-epipharynx (pl. 1, *F*) is a broad triangular structure which is kept rigid by three rodlike sclerotizations. At the tip of the labrum-epipharynx are two sclerotized trifid structures.

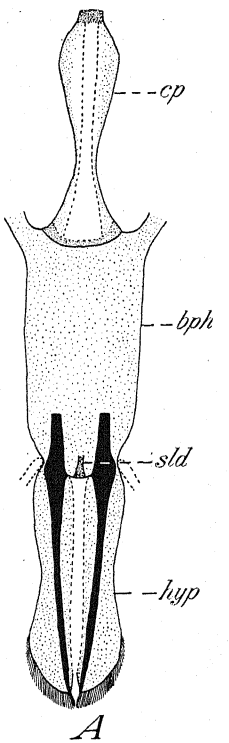
The hypopharynx (pl. 1, *A*) is a long narrow sclerotized rodlike structure the tip of which is armed with bristles. The salivary duct enters the proximal portion of the hypopharynx and is carried along the cephalic surface to the distal end.

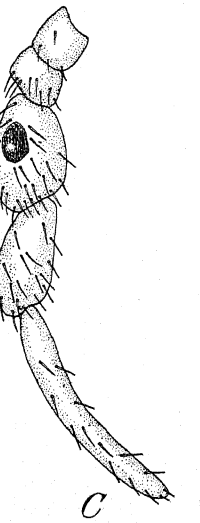
The basipharynx is a flattened channel formed by the union of the hypopharynx and epipharynx. The labium (pl. 1, *B*) is short and broad and the theca appears to consist of two separate parts but actually they are fused at the base. The large, free labella curve round and forward, and when the insect is biting surround the other mouth parts. The proximal parts of the labella are rigidly sclerotized while the distal portions are soft and bear numerous setae on the surface.

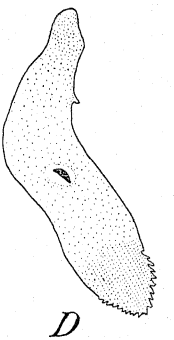
The maxillae lie along the outer margins of the labrum and hypopharynx. They are spear-shaped structures, the anterior face of which is flattened and is armed with teeth around its margin (pl. 1, *E*). The maxillary palps consist of five segments and are covered with numerous setae. The third segment bears a depressed saclike are which is probably a sensory vesicle (p. 1, *C*).

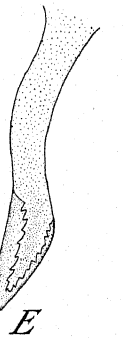
The mandibles are short, thin, swordlike structures fitted for piercing, and are armed with teeth on their anterior margins (pl. 1, *D*). Extending up the blade of each mandible from the marginal teeth are prominent striations. In the center of each mandible is a clear, crystallike area. The mandibles are so shaped that, for the greater part, the blades overlap between the labrum and the hypopharynx.

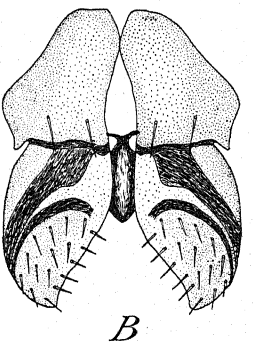
According to Snodgrass (*12*) the sucking apparatus of Diptera is the cibarium of orthopteroid insects and therefore would be called the cibarial pump. Also, the functional mouth aperture leading into the pump is actually not a true mouth. The true mouth occurs at the opening into the stomodaeum at the inner end of the pump. The cibarial pump of *Simulium nigroparvum* is triangular in section and consists of one dorsal sclerotized plate and two ventrolateral sclerotized plates. The pump is operated by muscles inserted on the plates (pl. 2, *C*).

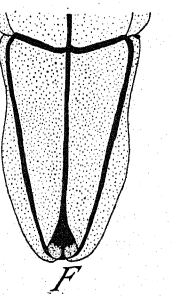




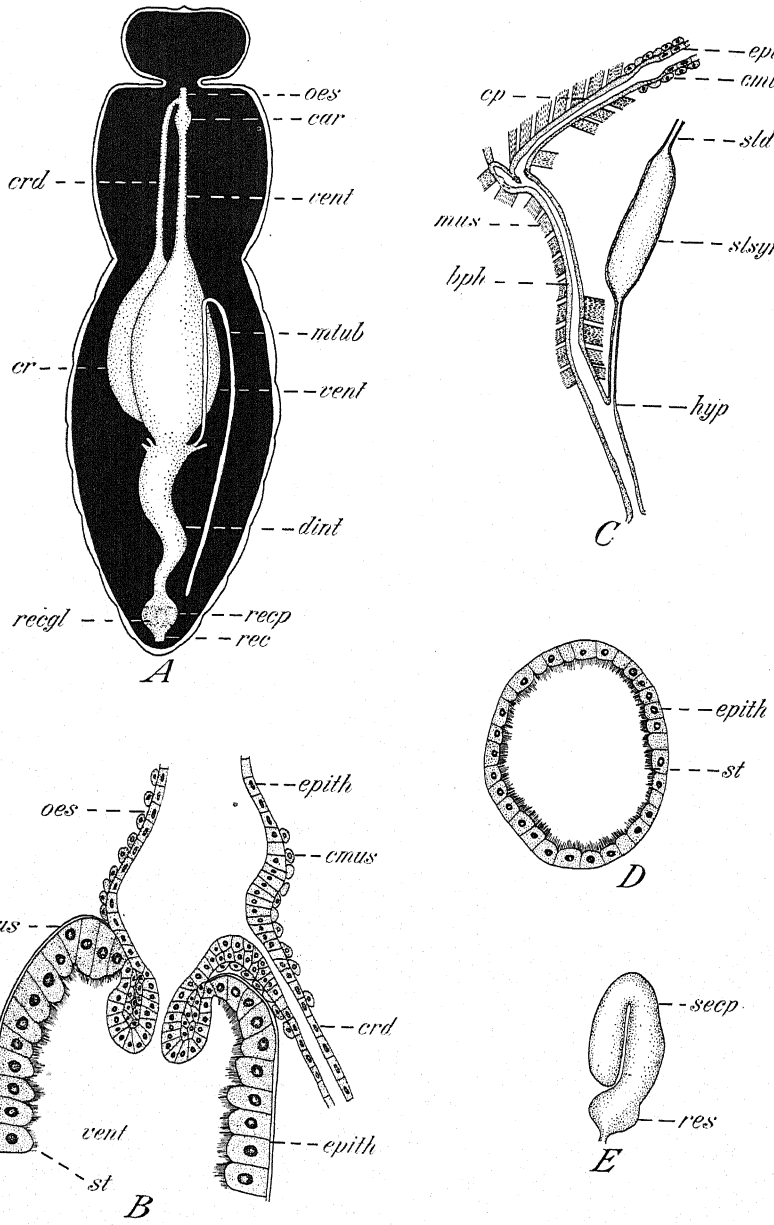








Mouth parts of *Simulium nigroparvum: A*, Hypopharynx, basipharynx, and cibarial pump; *B*, labium *C*, maxillary palp; *D*, mandible; *E*, maxilla; *F*, labrum epipharynx: *bph*, Basipharynx; *hyp*, hypopharynx *cp*, cibarial pump; *sld*, salivary duct.



Simulium nigroparvum: *A*, diagrammatic dorsal view showing the alimentary canal and Malpighian tubules; *B*, longitudinal section through cardiac valve; *C*, sagittal section through basipharynx and cibarial pump; *D*, cross section through anterior portion of ventriculus; *E*, salivary gland. *bph*, Basipharynx; *cmus*, circular muscle; *crd*, crop duct; *cr*, crop; *dint*, distal intestine; *epith*, epithelium; *hpy*, hypopharynx; *mtub*, malpighian tubule; *mus*, muscle; *cp*, cibarial pump; *oes*, oesophagus; *car*, cardia; *rec*, rectum; *recgl*, rectal gland; *recp*, rectal pouch; *res*, reservoir; *secp*, Secretory part; *sld*, salivary duct; *slsyr*, salivary syringe; *st*, striated margin of cell; *vent*, ventriculus.

SALIVARY GLANDS

The salivary glands, which are paired, lie in the anterior part of the thorax dorsad to the digestive tract. Each salivary gland is in the form of a U and is composed of two parts, viz, the anterior secretory part and the round saclike reservoir (pl. 2, *E*). A duct comes off from the reservoir of each gland. The two ducts from the salivary glands pass down, one on each side of the intestine, and unite beneath the oesophagus. The common duct leading from the point of junction expands to form the salivary syringe (pl. 2, *C*). From the salivary syringe the duct narrows and passes to the hypopharynx.

STOMODAEUM

The oesophagus, which is continuous with the cibarial pump, is a short, narrow tube that extends posteriorly between the head ganglia through the cervix, and joins the cardia of the ventriculus in the anterior part of the thorax. Within the thorax anterior to the cardia is the evaginated crop duct coming off the ventral side of the oesophagus and extending caudad beneath the ventriculus into the abdomen. Here it enlarges into an oblong thin-walled sac. The crop is capable of considerable distension and occupies most of the anterior part of the abdominal cavity of flies which have fed on sweetened water. However, no blood was found in the crop of flies which had fed on turkeys. The cardiac valve (pl. 2, *B*) marks the junction of the fore and mid intestine.

MESENTERON

The mid intestine, or ventriculus, is a straight tube of varying diameter. The anterior part is narrow and tubelike and extends along the median line of the body to the abdomen where it expands over the crop, the degree of expansion depending on the amount of blood the fly has consumed. The mid intestine is marked at its anterior end by the cardiac valve and at the posterior end by the pyloric valve. The posterior portion of the mid intestine narrows slightly where it joins the hind intestine.

PROCTODAEUM

The hind intestine is marked anteriorly by the pyloric valve and posteriorly by the rectum. At the point of junction of the mid and the hind intestine four Malpighian tubules arise, one pair on each side of the intestine. They are of considerable length and follow an irregular course through the abdominal cavity.

The anterior part of the hind intestine is a slightly tapering tube which may or may not be curved, depending on the development of the ovaries. This is known as the distal intestine. The posterior part of the hind intestine forms a flask-shaped sac called the rectal pouch, which contains six rectal glands. The rectal glands are arranged in a circle around the opening of the distal intestine and are visible through the thin walls of the rectal pouch.

The rectum is a short tube which extends from the rectal pouch to the anus.

95981—38——4

HISTOLOGY OF THE ALIMENTARY TRACT

MOUTH PARTS AND BITING APPARATUS

The mouth parts, being true appendages of the head, have the same structure as the body wall. The chitinous lining of the food channel is heavy and rigid. It is overlain by a very thin layer of epithelium; and bands of muscles extend from the body wall to the labrum-epipharynx and the labium-hypopharynx.

The walls of the basipharynx are heavy, chitinous structures overlain by a thin layer of epithelium. Muscle bands are attached to its anterior face.

SALIVARY GLANDS

The salivary glands are tubular and are composed of a single layer of cells whose bases rest on the basement membrane. The elongated secretory portion of each gland is composed of large cuboidal cells and the reservoir part is made up of small flattened cells (pl. 3, *D*). From the reservoir part of each gland a slender duct arises, which unites with that of the other gland to form the common salivary duct. A short distance from the point of junction, the duct expands to form the salivary syringe, whose walls are strengthened by riblike sclerotizations (pl. 2, *C*). The walls of the salivary duct are composed of a chitinous intima and a very thin layer of epithelium. No muscle layer was found on either the glands or the duct.

STOMODAEUM

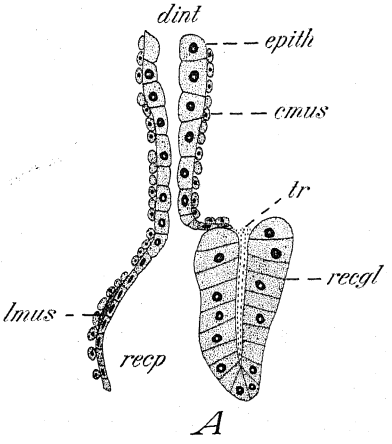
The oesophagus and its diverticulum, the crop, are true stomodeal structures and show a similar histological structure. The chitinous intima is so thin that it cannot be detected in most sections. Also, a thin layer of epithelium is found in all parts and is composed of flattened cells joined at their bases to a basement membrane. Covering the epithelium is a layer of circular muscle fibers. In the crop both the epithelial and the muscle layer are extremely thin.

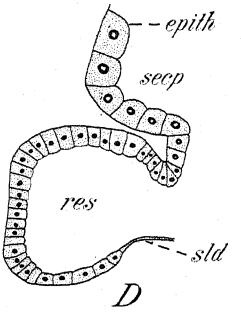
The oesophagus extends well into the ventriculus and is represented by a single layer of epithelial cells. The inability to distinguish a chitinous intima makes the determination of the limits of the fore intestine difficult. It is also difficult to determine the ectodermal from the endodermal tissue in sections through the cardiac valve.

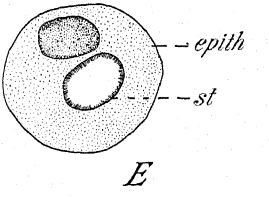
MESENTERON

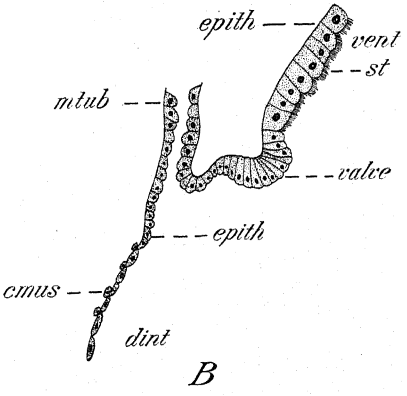
The mid intestine, or ventriculus, differs from the fore and the hind intestine in histological structure. The inner and most conspicuous layer of the stomach is that of the epithelium, the bases of whose cells rest on the basement membrane (pl. 2, *D*). Following the basement membrane is a very thin layer of circular muscles and over these are scattered longitudinal muscles. The cells of the epithelium are columnar (pl. 3, *G*) and are sometimes thrown into irregular folds. The epithelium of the posterior part of the stomach is variable in thickness and, when the gut is distended with food, is only about one-third the thickness of the normal epithelium (pl. 3, *C*). The epithelial cells in all parts of the mid intestine show a striated inner margin.

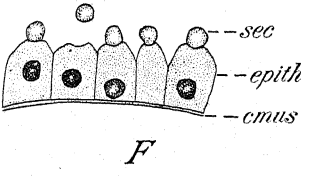
The epithelium of the mid intestine functions in the secretion of digestive fluids. The secretion is merocrine inasmuch as the entire cell does not burst. Sections taken through the stomach of an unfed

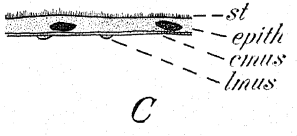


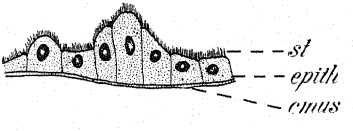












Simulium nigroparvum. *A*, Longitudinal section through a portion of the distal intestine and one rectal gland; *B*, longitudinal section through pyloric region; *C* longitudinal section through the ventriculus of an engorged fly showing reduced epithelium; *D*, longitudinal section through salivary gland; *E*, cross section through Malpighian tubule; *F*, longitudinal section through ventriculus of an unfed fly; *G*, longitudinal section through ventriculus showing cuboid epithelium. *cmus*, Circular muscle; *dint*, distal intestine; *epith*, epithelium; *lmus*, longitudinal muscle; *mtub*, malpighian tubule; *recgl*, rectal gland; *recp*, rectal pouch; *res*, reservoir; *sec*, secretion; *secp*, secretory part; *sld*, salivary duct; *st*, striated margin of cell; *tr*, tracheal branch; *vent*, ventriculus.



fly show a very active secretion taking place (pl. 3, *F*). The inner end of the cells bud off and there is no rupturing of the cell wall. During the process of secretion the epithelial cells do not show the striated border.

PROCTODAEUM

The hind intestine is marked anteriorly by the pyloric valve. This valve is formed by a ring of epithelial cells at the end of the mid intestine. These cells, unlike those found in the stomach, have no striated border. The lip of the valve encircles the intestine just anterior to the opening of the Malpighian tubules (pl. 3, *B*). The cells of the Malpighian tubules are of the characteristic cuboidal shape and are continuous with the epithelium of the hind intestine. A short distance from the point of attachment, the cells of the Malpighian tubules are narrow and elongated with conspicuous nuclei. Their inner margins are distinctly striated (pl. 3, *E*).

The epithelium of the distal intestine consists of flattened cells, which are raised into folds in the posterior part. Covering the epithelium is a layer of circular muscles which forms a continuous layer at the posterior end. As in the fore intestine, the intima in the distal intestine is too thin to be readily demonstrated.

The walls of the rectal pouch are extremely thin and the epithelium is greatly reduced except where it forms the rectal glands. Both muscle layers are very thin and the rectal glands are readily visible through the walls of the rectal pouch. The rectal glands (pl. 3, *A*) are composed of large cells with prominent nuclei. The lumen of each gland is occupied by a tracheal branch. The chitinous intima is very thin and not easily demonstrated in the rectal pouch.

The posterior end of the rectal pouch narrows abruptly to form the rectum. Here both muscle layers as well as the epithelium are well developed and the chitinous intima lining the rectum can easily be detected.

LITERATURE CITED

(1) BLACKLOCK, D. B.
1926. THE DEVELOPMENT OF ONCHOCERCA VOLVULUS IN SIMULIUM DAMNOSUM. Ann. Trop. Med. and Parasit. 20: 1–48, illus. [Abstract in Rev. Appl. Ent. (B) 14: 62–63, 1927.]

(2) ———
1926. THE FURTHER DEVELOPMENT OF ONCHOCERCA VOLVULUS LEUCKART IN SIMULIUM DAMNOSUM THEOB. Ann. Trop. Med. and Parasit. 20: 203–218, illus. [Abstract in Rev. Appl. Ent. (B) 14: 167–168, 1927.]

(3) CAMERON, A. E.
1922. THE MORPHOLOGY AND BIOLOGY OF A CANADIAN CATTLE-INFESTING BLACK FLY, SIMULIUM SIMILE MALL. (DIPTERA SIMULIIDAE). Canada Dept. Agr. Bull. 5, n. s., (tech.), 26 pp., illus.

(4) DIMMOCK, GEORGE.
1881. ANATOMY OF THE MOUTH-PARTS AND THE SUCTORIAL APPARATUS OF CULEX. Psyche 3: 231–241, illus.

(5) EMERY, W. T.
1913. THE MORPHOLOGY AND BIOLOGY OF SIMULIUM VITTATUM AND ITS DISTRIBUTION IN KANSAS. Kan. Univ. Sci. Bull. 8: 323–362, illus.

(6) HUNGERFORD, H. B.
1913. ANATOMY OF SIMULIUM VITTATUM. Kan. Univ. Sci. Bull. 8: 365–382, illus.

(7) JOBLING, B.
1927. THE STRUCTURE OF THE HEAD AND MOUTH PARTS IN CULICOIDES PULICARIS, L., (DIPTERA NEMATOCERA). Bull. Ent. Research 18: 211–236, illus.

(8) JOHNSON, E. P., and UNDERHILL, G. W.
1937. A BLOOD DISEASE OF TURKEYS. South. Planter 98 (1): 34, 39; (9): 32.
(9) KELLOGG, VERNON L.
1899. THE MOUTHPARTS OF THE NEMATOCEROUS DIPTERA, II–V. Psyche 8: 327–330, 346–348, 355–359, 363–365, illus.
(10) O'ROKE, E. C.
1934. A MALARIA-LIKE DISEASE OF DUCKS CAUSED BY LEUCOCYTOZOON ANATIS WICKWARE. Mich. Univ. School of Forestry and Conserv. Bull. 4, 44 pp., illus.
(11) SMART, JOHN.
1935. THE INTERNAL ANATOMY OF THE BLACK FLY, SIMULIUM ORNATUM MG. Ann. Trop. Med. and Parasit. 29: 161–170, illus.
(12) SNODGRASS, R. E.
1935. PRINCIPLES OF INSECT MORPHOLOGY. 667 pp., illus. New York and London.

SEASONAL VARIATIONS IN THE CARBOHYDRATE AND NITROGEN CONTENT OF ROOTS OF BEARING PECAN TREES [1]

By C. L. SMITH, *associate physiologist*, and J. G. WAUGH, *assistant physiologist*, *Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture* [2]

INTRODUCTION

In the pecan (*Hicoria pecan* (Marsh.) Britton), as in other perennial plants, the early growth of the tree in spring is determined largely by the food reserves that have accumulated during the previous growing season and are made available in spring. These food reserves play a part in developing resistance to cold during winter and are utilized for new shoot and root growth, initiation of pistillate flowers, production of catkins and pollen, and the initial development of the nut.

Previous analyses of roots of top-worked pecan trees [3] indicated that there was considerable storage of food reserves in the roots and that a heavy nut crop materially affected the seasonal variation of the stored constituents. Since the pecan generally bears only in alternate years, and in view of the importance of food reserves in the growth, development, and fruiting performance of the tree, it seemed desirable to obtain some information on the proportionate seasonal accumulation of these food reserves in the roots of bearing pecan trees and the effect of a crop on their accumulation.

REVIEW OF LITERATURE

LeClerc du Sablon (*12, v. 16*)[4] analyzed the roots, stems, and leaves of various deciduous trees, including the chestnut, pear, and peach, for sucrose, starch, hemicellulose, and moisture. In general, he found that the total carbohydrate reserves in the roots pass through a maximum in autumn at the time of leaf fall, diminish a little in winter, and diminish greatly during growth in the spring. After growth has subsided and assimilation is at its height, reserve carbohydrates increase and attain their maximum again in autumn. The carbohydrate content of the roots was higher than that of the stems and showed more variation, indicating that the roots are more important storage organs than the stems. The extensive variation of hemicellulose indicated that it may be an important reserve. The variation of sucrose was relatively slight, and LeClerc du Sablon concludes that sucrose is a less important reserve material than the form in which other carbohydrates become mobile and assimilable.

Later LeClerc du Sablon (*12, v. 18*) worked with several evergreen trees. In all cases he found that the total carbohydrates in the roots

[1] Received for publication December 28, 1937; issued September 1938.
[2] The writers wish to express their appreciation to L. D. Romberg for determining the dry matter and assisting in preserving samples during the progress of this investigation.
[3] SMITH, C. L., HAMILTON, J., THOR, C. J. B., and ROMBERG, L. D. EFFECTS OF VARYING DEGREES OF SEVERITY IN HEADING BACK LARGE PECAN TREES IN TOP-WORKING ON THEIR SUBSEQUENT ROOT COMPOSITION AND TOP DEVELOPMENT. [In manuscript.]
[4] Italic numbers in parentheses refer to Literature Cited, p. 459.

decreased during leaf formation and new shoot development and increased after this growth was completed, although the growth periods occurred at different times of the year. He also analyzed the stems, roots, and leaves of the evergreen oak and Japanese spindle tree for nitrogen and found that in the roots it decreased in spring during new leaf formation, passed through a minimum in summer, and increased toward winter. He concluded that in spring nitrogen moves from the roots and stems to the newly formed leaves and, when growth ceases, continues to be absorbed and is stored, thereby increasing in late summer and toward winter.

Butler et al. (*2*) analyzed different parts of the apple tree and found that the reserves were stored chiefly as starch and sucrose, the roots and branches containing relatively more starch than the trunk. Davis (*6*) found that the roots and trunks of nonbearing Sugar prune trees contained much more starch than those of bearing trees, and that the starch concentration in the roots was much greater than in the trunks. He states that it is reasonable to suppose that root growth is suppressed when the demand for carbohydrates above ground becomes great, and suggests that fruit trees, which have a heavy annual crop that remains on the trees most of the summer, may experience a shortage of carbohydrates for the roots. He also noted that starch was the most variable of the carbohydrates. Cameron (*3*), working with young orange trees, noted a minimum of starch in the roots in August, then a gradual increase to a maximum in early spring, and a gradual decrease to a minimum again in August. An inverse relation between starch and moisture was indicated. For the greater part of the year, the starch content in the roots was higher than in the leaves, branches, or trunk, and the greatest starch fluctuation occurred in the root bark. Murneek (*16*), investigating the carbohydrate storage of bearing apple trees, noted the unusually high starch and sugar content of the roots in autumn. He concluded that the underground parts of the apple tree serve as storage organs for carbohydrates, primarily starch. His data indicate that there is an increase in total sugars and a slight decrease in hemicellulose, starch, and total carbohydrates in the roots from early November to late December.

The importance of hemicellulose as a reserve material has been stressed by a number of investigators working with parts of the tree other than the roots. Schellenberg (*18*), in a summary of such investigations, concludes that hemicellulose serves as a reserve material and its deposition enables the plant to survive the long rest periods (winter or dry period) into which it is forced at times. Tottingham et al. (*21*) and Murneek (*14, 15*), working with apple spurs, noted extreme fluctuations of hemicellulose and emphasized its significance as an available form of carbohydrate in the spur. Its accretion in the fruit reached a maximum in midsummer and then decreased rapidly, thus indicating that hemicellulose must be a source of sugars for the flesh of the apple. Hooker (*11*) found an extensive variation in hemicellulose in apple spurs, which seems to indicate that it functions as a reserve material. Murneek and Logan (*17*), investigating the autumnal migration of nitrogen and carbohydrates in the apple tree, concluded that with the onset of cold weather starch and possibly other more complex carbohydrates are hydrolyzed simultaneously into sugars in all peripheral

regions of the tree. They state that modifications induced by weather often are of greater amplitude than possible seasonal trends.

Finch (*7*, *8*) concluded that for best filling of pecan nuts in Arizona the vegetative growth of trees must be checked during the early summer to provide conditions for carbohydrate storage during the summer months. He found that starch was formed in the current-season shoots in summer but disappeared completely during the nut-filling period. This emphasizes the importance of an abundant supply of carbohydrates for conversion to fats in nut filling.

Finch and Van Horn (*9*) found that starch storage in bearing pecan shoots reached a maximum shortly before nut filling began, and then decreased; whereas a comparable decrease did not occur in vegetative nonfruiting shoots. In the latter starch continued to accumulate, reaching a maximum in early fall. These authors conclude that the starch from the fruiting shoots is used in nut filling. Thor and Smith (*20*) have shown that most of the oil in pecan kernels is formed within a period of 4 to 6 weeks and that most of the carbohydrate material for its formation must be brought in from outside the nut during this period.

The maintenance of healthy foliage on the fruit tree has been emphasized by Heinicke and Hoffman (*10*) and other workers. Sitton (*19*) ringed bearing shoots of the pecan in early August after the shells of the nuts had begun to harden, but before any considerable filling had taken place, and noted that the best filling was obtained in nuts on shoots that bore the largest number of leaves per nut. Crane et al. (*5*) conclude that the almost universal tendency of pecan trees to bear more or less irregular crops from year to year cannot be called either biennial or alternate bearing and that without doubt the controlling factors are the nutritional condition and previous performance of the tree. Their data indicate that nut thinning may be expected to increase the annual yield per tree by maintaining a more nearly optimum nutritional condition in the tree at all times. Smith et al.,[5] in studies on top-working pecan trees, note the value of the leaf area in the functioning of the tree. Chemical analyses of the roots showed that with severe heading the balance of the carbohydrate reserves in the tree is disturbed to a greater degree than in trees with less severe topping. As the top is rebuilt the amount of these materials and their functions become more nearly like those of the normal tree. Crane and Hardy (*4*) have shown that cultural treatments, such as pruning or applications of nitrogen, which stimulate shoot growth with subsequent increase in leaf area, are of great importance in determining the ultimate size of pecan nuts and the degree to which they are filled.

MATERIALS AND METHODS

Since the pecan is frequently an alternate bearer, the progress of this investigation was facilitated by the use of trees in an alternate bearing condition during "off" and "on" years. During the first season of the experiment there was no nut crop; in the second season the crop was large; in the third season the crop was very small.

Seventy-five healthy seedling trees about 45 years old, growing in a cultivated river-bottom field about 15 miles from Austin, Tex.,

[5] SMITH, C. L., HAMILTON, J., THOR, C. J. B., and ROMBERG, L. D. See footnote 3

were selected and divided into 15 comparable groups of 5 trees each. The average diameter of the trees was about 25 inches. Composite root samples for chemical analysis were taken from these groups in rotation in time intervals ranging from 2 weeks to approximately 1 month. Owing to the death of the farm owner and subsequent litigation of ownership of the property, it was necessary to discontinue the investigations from October 1935 to August 1936. A summarized description of the condition of the trees during the course of the 3 years is given in the last column of table 1 (p. 457).

SAMPLING METHODS

Samples of lateral roots with diameters ranging from three-eighths to one-half inch were taken, brought immediately to the laboratory, chopped fine, and mixed thoroughly. Samples to be dried were killed by placing them in an oven at 100° C. for 1 to 2 hours and were then dried to constant weight in a vacuum oven. Two 50-g portions were transferred immediately to 500-ml Erlenmeyer flasks with sufficient boiling 95-percent alcohol to give a final alcohol concentration of 80 percent by weight. The flasks were then closed with short-stemmed funnels and heated 30 minutes on a water bath kept at 90° to 95°. The flasks were stoppered while still hot and stored in the dark until the samples were used for carbohydrate analyses.

ANALYTICAL METHODS

Whenever the nature of the material permitted, the official methods of the Association of Official Agricultural Chemists (*1*) were employed.

Dry Matter

Samples that had been weighed into aluminum cans and placed in ovens at 100° C. for 1 to 2 hours were transferred to a vacuum oven and dried to constant weight at a temperature of 80° and a pressure of less than 1 mm of mercury. The resulting dry material was ground in a drug mill or food chopper and stored in glass bottles, and was redried under the same conditions before it was used in further analyses.

Total Organic Nitrogen

No analyses were made for nitrate or nitrite nitrogen since previous analyses had shown no appreciable amounts of these forms of nitrogen in pecan roots. The procedure for determination of total organic nitrogen followed closely the Kjeldahl-Gunning-Arnold method except for the use of copper wire as the digestion catalyst. In general 2-g portions of the dried material were used. The preliminary drying of the sample at 100° C. for 1 to 2 hours has been shown by Link and Schulz (*13*) to have little or no effect on the total nitrogen content of plant tissues.

Sugars

The alcohol-preserved samples were transferred to large Soxhlet extractors with 80-percent alcohol and extracted for 22 to 24 hours on a water bath kept at 95° to 99° C. The alcohol extract was distilled almost to dryness in vacuo at temperatures not above 60°. The residue was taken up with water, treated with 25 cc of basic

lead acetate to clear the solution, and made up to a volume of 250 ml. The lead acetate solution used for clearing was made up of 500 g of basic lead acetate in 1 liter of solution. The cleared filtrate was deleaded with anhydrous disodium phosphate.

The Munson-Walker method was used for the determination of sugars in aliquots of the lead-free filtrate. Reduced copper was determined by the volumetric permanganate method. For total sugars, inversion was accomplished by means of acid at room temperature as directed in official methods (*1, p. 187,* [*23c*]). Reducing-sugar values were calculated as invert sugar and nonreducing sugars as sucrose.

Starch by Diastase

The alcohol-insoluble residues were transferred to tared aluminum cans, dried in an air oven at 100° C., and weighed. After being ground in a drug mill the material was stored in aluminum cans in a desiccator until ready for analysis.

The official method for determination of starch in feeding stuffs by diastase with subsequent acid hydrolysis (*1, p. 120,* [*23*]) was used with the exception that a 0.5-percent solution of taka diastase was substituted for the malt extract. Preliminary extractions with cold water were eliminated since sugars had already been extracted with alcohol.

Starch by Direct Acid Hydrolysis

Alcohol-insoluble residues were used as in the diastase method. The official methods (*1, p. 119,* [*21*]) were used. As before, no preliminary extractions with cold water were necessary.

Those carbohydrates that are not hydrolyzed by the taka-diastase method for starch determination, but are hydrolyzed in the direct acid hydrolysis method, are designated as hemicelluloses. The hemicellulose values were obtained by subtracting the starch by taka-diastase values from the corresponding values for starch by direct acid hydrolysis.

EXPERIMENTAL DATA

Since no investigations were made on the variability of the constituents in comparable samples obtained at the same time, no particular emphasis can be attached to the analytical data for any individual sample, and the results must be viewed from the standpoint of trends. The analytical data are presented in figure 1.

DRY MATTER

The percentage of dry matter in the roots was higher in the summer and fall of 1934 than at any other time during the experiment. This was probably due to a deficiency of soil moisture during an extreme drought which lasted until November. After the drought was broken by rains the percentage of dry matter in the roots decreased rapidly until about January 1935. Between January and September there was only a slight decrease in dry matter, but from the middle of September to the latter part of October there was a considerable decrease. From August 1936 to February 1937 the dry matter fluctuated considerably between sampling dates, but showed no consistent increase or decrease.

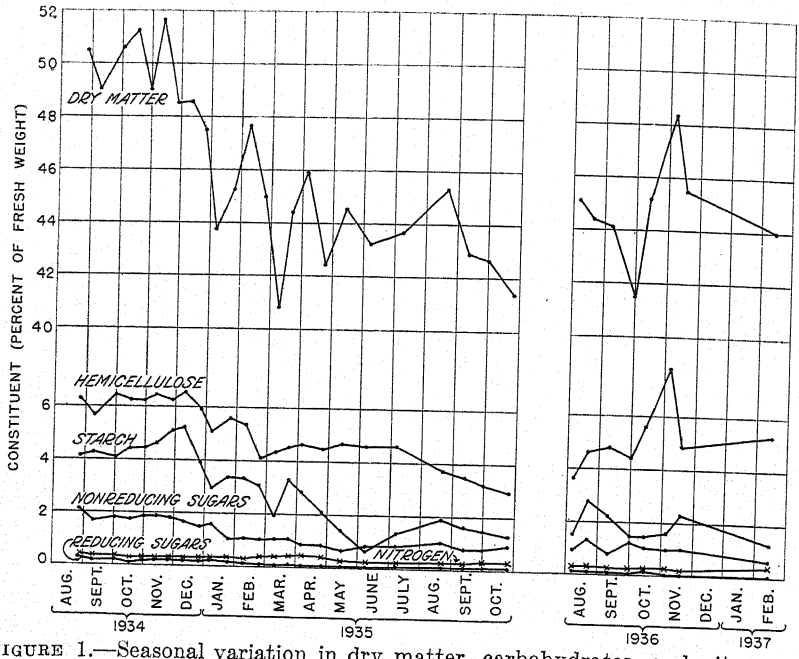


FIGURE 1.—Seasonal variation in dry matter, carbohydrates, and nitrogen in roots of pecan trees.

The following tabulation shows the monthly rainfall record at Austin, Tex., from January 1, 1934, to February 20, 1937, inclusive:

Year and month:	*Rainfall (inches)*
1934	
January	7. 43
February	5. 01
March	2. 37
April	3. 31
May	. 90
June	. 32
July	. 39
August	. 24
September	2. 61
October	. 32
November	3. 40
December	4. 59
1935	
January	. 95
February	2. 96
March	1. 20
April	2. 08
May	9. 21
June	9. 71
July	1. 44

Year and month—Continued.	*Rainfall (inches)*
1935—Continued	
August	. 24
September	8. 79
October	1. 65
November	. 85
December	2. 84
1936	
January	0. 39
February	1. 70
March	1. 52
April	. 66
May	8. 15
June	3. 30
July	9. 25
August	2. 90
September	5. 22
October	2. 63
November	2. 30
December	1. 88
1937	
January	2. 43
February	. 12

This record indicates that the roots had an abundant moisture supply throughout the experiment except in the summer and fall of 1934. The rainfall was low in July and August of 1935 but there was sufficient rain in June to carry the trees through this period without a moisture deficiency.

SUGARS

The concentration of reducing sugars was very low throughout the experiment and showed no consistent seasonal variations. The nonreducing sugars were present in appreciable quantities and fluctuated as starch did below but to a lesser extent. The highest concentration occurred in the late summer and fall of 1934. There was a decrease during the winter and spring, due in part to the decrease in dry matter. From May 13 to July 6 there was an appreciable increase in the nonreducing sugars, after which their concentration remained practically constant until October 25. On August 6, 1936, the concentration of these sugars was at the same level as in October 1935, but it increased considerably during August and then remained practically constant until November 18. Between the latter date and February 16, 1937, there was a material decrease similar to that in the winter of 1934–35.

STARCH

The concentration of starch increased slightly from August to December 1934, decreased rapidly to January 1935, and then decreased more slowly to June 7. There was no nut crop on the trees in 1934, and the increase in starch content of the roots was probably due to the photosynthetic activity of the leaves and translocation of carbohydrates to the roots where they were stored as starch. This seems to be further indicated by the comparatively high concentration of nonreducing sugars present in the roots during this period. From December 1934, after most of the leaves had fallen or had become inactive from frost, there was a rapid decrease in concentration of starch to January 1935. A decrease in carbohydrates in roots after leaf fall has been noted by other investigators (*12*, *16*) and indicates a translocation of these substances to the aerial portions of the tree. From January 7 to March 22 the starch showed only slight changes except for one low value on March 8. After March 22 the starch decreased rapidly, reaching a minimum on June 7. During the latter period the initiation of growth took place, shoots and leaves developed, the trees blossomed, and the development of the nuts was begun. All these processes create heavy demands upon the tree for carbohydrates. The leaves during most of this period are immature and small, and their total photosynthetic activity is very low and therefore inadequate to supply the necessary carbohydrates for the rapid growth and fruiting activities. It is interesting to note that the concentration of the hemicellulose remained practically constant during this period notwithstanding the fact that from December 10 to February 23 it decreased in about the same proportion as starch.

From June 7 to August 20, 1935, there was a considerable increase in the starch. During this period the shucks and shells of the nuts were being developed, but other vegetative growth was probably nearly completed for the season. The leaves were probably at their most efficient stage for photosynthesis and therefore were able to produce a greater amount of carbohydrates than was required for tree activities, some of the surplus being stored in the roots as starch.

From August 20 to October 25, 1935, the starch content of the roots decreased slightly. About September 1 the period of nut filling begins and the demand for carbohydrates during this period has been shown to be very great (*20*). This is especially true when the trees are carrying a large crop of nuts, as was the case here. Both starch and

hemicellulose concentrations decreased during the nut-filling period from September 1 to the latter part of October. A part of this decrease was due to a decrease in the dry matter of the roots, but calculations show that both starch and hemicellulose decreased in greater proportion than the dry matter. Thus it seems probable that some of the hemicellulose as well as the starch was utilized in the nut-filling process. No doubt the photosynthetic activity of the leaves was high during the nut-filling period, but with a heavy crop of nuts on the trees the demand for carbohydrates for oil formation was greater than could be supplied by photosynthesis, and some of the reserves in the tree were used.

After the 9-month interval from October 25, 1935, to August 6, 1936, during which no samples were taken, the starch concentration showed a slight increase, which may be accounted for by the increase in the percentage of dry matter during this period. However, the starch concentration was still not so high in proportion to the dry matter as it was in 1934. The starch fluctuated considerably during the fall of 1936 but showed no consistent increase. On February 16, 1937, when the last samples were taken, the starch was low, amounting to 1.26 percent of the fresh weight. The nut crop was small in 1936 and therefore made much less demand for carbohydrates for nut filling than the large crop of 1935, but the starch content of the roots on the average was only slightly higher than for the corresponding period in 1935. Conditions were very favorable for vegetative growth in 1936, however, and the starch was probably utilized for this purpose.

HEMICELLULOSE

The percentage of hemicellulose was at its highest level in the summer and fall of 1934, when the dry matter was at its maximum. Its fluctuation was almost proportional to that of the dry matter from August 1934 to January 1935. From January to October, however, the percentage of hemicellulose shows a greater proportional decrease than the percentage of dry matter. This indicates that there was an actual decrease in the amount of hemicellulose in the roots and that only a part of the variation in its concentration was due to changes in the concentration of dry matter. The trees bloomed heavily in the spring of 1935 and matured a large crop of nuts. Therefore it is possible that some of the hemicellulose was utilized in the development and filling of the nuts. From October 1935 to August 1936 the percentage of hemicellulose had increased a little but not in proportion to the increase in dry matter. From the latter part of 1936 to February 1937 the hemicellulose increased gradually, but at the end of this period it was still somewhat lower in proportion to dry matter than in the fall of 1934.

NITROGEN

The percentage of total organic nitrogen decreased slightly from August 27, 1934, to January 7, 1935 (fig. 1 and table 1). This decrease may be accounted for by the decrease in the concentration of dry matter. There was a gradual increase in the nitrogen concentration from January 7 to April 5, probably because the roots absorbed more nitrogen than was translocated away. There was little growth activity in the tree during this period, while at the same time soil moisture and probably other factors were optimum for the absorption of nitrogen.

TABLE 1.—*Seasonal variation in total organic nitrogen in pecan roots and growth and fruiting condition of trees*

Sample No.	Sampling date	Total organic nitrogen	Growth and fruiting conditions
	1934	*Percent* [1]	
1	Aug. 27	0.38	Foliage on all trees apparently fully mature and in healthy condition. No apparent extension of shoot growth. No nut crop on trees.
2	Sept. 10	.39	
3	Oct. 2	.26	
4	Oct. 16	.31	
5	Oct. 30	.29	
6	Nov. 12	.34	About 3 percent of leaves have dropped.
7	Nov. 27	.30	Trees have lost about 40 percent of foliage and owing to frost leaves are turning yellow and dropping rapidly.
8	Dec. 10	.32	
9	Dec. 26	.33	Defoliation complete.
	1935		
10	Jan. 7	.32	Trees dormant.
11	Jan. 23	.39	
13	Feb. 8	.35	
15	Feb. 23	.40	
17	Mar. 8	.40	
19	Mar. 22	.45	Buds bursting into growth on a few trees.
21	Apr. 5	.52	Most trees well into growth and a few almost in full leaf. Heavy pistillate bloom.
22	Apr. 24	.42	Pollen shedding very active.
23	May 13	.34	Shoot growth well under way and active.
24	June 7	.35	Shoot growth nearly complete. Large crop of nuts set.
25	July 6	.27	Shoot growth complete and foliage mature.
27	Aug. 20	.32	Nuts in "watery" stage.
29	Sept. 12	.33	Nuts in "dough" stage.
31	Sept. 30	.37	Nuts beginning to ripen.
32	Oct. 25	.33	Nuts mature and dropping from trees. Foliage dropping rapidly.
	1936		
35	Aug. 6	.33	Foliage full grown and in good condition. Extension of shoot growth apparently continuing through most of this period. Nut crop very small.
36	Aug. 20	.31	
37	Sept. 9	.34	
38	Oct. 1	.34	
39	Oct. 13	.32	Nuts ripening.
40	Nov. 6	.37	Leaves beginning to fall. Nuts fully mature and dropped.
41	Nov. 18	.32	Trees almost completely defoliated.
	1937		
42	Feb. 16	.40	Trees dormant.

[1] Percentage based on fresh weight.

From April 15 to May 13, 1935, the nitrogen decreased to about its 1934 level, after which it remained practically constant for the remainder of the experiment, except that it had increased appreciably between November 18, 1936, and February 16, 1937.

The decrease in nitrogen in April and the first part of May may be ascribed to the intensive vegetative activity of the trees at this time. The initiation of shoot growth and the development of blossoms took place during this period and consequently created a heavy demand for nitrogen. Conditions were no doubt favorable for the absorption of nitrogen at this time, but the demand was probably greater than could be supplied by root absorption, and some of the reserve nitrogen from the tissues was used in the growth functions. After the intensive vegetative activity of the tree was over there was a large crop of nuts to be developed and filled; therefore the nitrogen did not increase again during that season. In the fall of 1936 the nut crop was light, but conditions for vegetative growth of the trees were more favorable than in 1935 and the growth extended later into the season, so that all the nitrogen absorbed was required in growth processes. After the growth season was over the nitrogen increased appreciably during the winter. In 1934 the low level of nitrogen in the roots may have been due to the extremely dry weather, which created unfavorable conditions for absorption of nitrogen by the roots.

DISCUSSION

Starch was the most variable constituent determined in the roots of pecan trees. The data indicate that it increases from early summer to a maximum in late fall and decreases to the summer minimum, but the levels of the maxima and minima seem to be determined by the growth and fruiting conditions during the time. Both growth and fruiting in the pecan are exhaustive processes, as has been found in other trees by Murneek (*14*), Davis (*6*), and other investigators. The starch in pecan roots was built up to a high maximum concentration in the fall of 1934, when there was no nut crop, and the vegetative growth was at a standstill owing to a severe drought. With the intensive spring growth and blossoming of the trees in 1935 the starch decreased to a very low level and did not increase during the summer and fall because of the great demand for carbohydrates in filling the large crop of nuts. There was also a decrease in the hemicellulose content of the roots during the nut-filling period. This indicates that both starch and hemicellulose may function as carbohydrate reserves and are used in the filling of the nuts or in other processes when the demand is sufficiently great.

It is not known how low a level the starch concentration reached in the fall of 1935, since no samples could be taken after October 25 until August 6 of the following year. However, on the latter date the starch concentration was still relatively low and did not increase materially during the autumn notwithstanding the fact that the nut crop was very small. Conditions were very favorable for vegetative growth throughout the season, and the failure of the starch to increase in the autumn can be explained only on the assumption that tree growth continued throughout the fall, thereby utilizing the carbohydrates, in this process and in the filling of the small crop of nuts, as fast as they were synthesized.

In the spring of 1936 and in the spring of 1937 the bloom was light and the starch in the autumn preceding each of these seasons was at a low concentration, whereas in the fall of 1934 the starch reached a high concentration which was followed by a very heavy bloom in the spring of 1935. Whether the lack of reserve carbohydrates, as indicated by starch, was the cause of the small bloom in the 2 later years is not known, but this same condition has been found in other trees (*6*, *11*) and seems to be either directly or indirectly concerned with fruiting. The fact that the pecan seldom, if ever, bears two large crops in successive years and that fruiting is a very exhaustive process indicates that the amount of storage carbohydrates, especially starch, is a very important factor in fruiting whether the effect be direct or indirect. Davis (*6*) found that the starch content of roots of nonbearing Sugar prune trees was much higher than that of roots of bearing trees, and concludes that storage carbohydrates may be essential for fruit-bud differentiation or that other factors may cause fruit-bud differentiation because of their relationships to the storage carbohydrates.

The nonreducing sugars were present in appreciable quantities and fluctuated with the starch, although to a lesser extent. The wide variations in concentration of these sugars may indicate that they function as storage reserves. However, the fact that they fluctuated so nearly with the starch may indicate that they are labile forms and that their concentration is largely dependent upon that of the starch.

Although the concentration of nitrogen was relatively low throughout the experiment, the data indicate that the amount of nitrogen in pecan roots is governed partly by conditions favorable to absorption and partly by the nitrogen requirements for tree growth and fruiting. In the latter part of 1934 the nitrogen concentration was low, but it increased from January to April in 1935. The summer and fall of 1934 were extremely dry and the soil moisture was doubtless a limiting factor in the absorption of nitrogen. From January until April 1935, however, soil moisture was adequate for absorption, and the trees were dormant and therefore required very little nitrogen. With the initiation of spring growth and blossom development the demand for nitrogen was great and probably could not be met by root absorption; hence the nitrogen decreased in the roots. The heavy nut crop of 1935 made a considerable demand for nitrogen in nut filling, and the nitrogen did not increase during that time. However, from November 1936 to February 1937, when the trees were dormant, the nitrogen again increased materially as in the first part of 1935.

SUMMARY

Composite samples of lateral roots of pecan trees of bearing age were collected at intervals during an "off" and an "on" year and analyzed for the principal carbohydrates and total organic nitrogen.

The starch concentration tended to reach a maximum in late fall, after which there was a winter decrease and then a further decrease in spring to a minimum in early summer. The amount of starch in the roots for any period was apparently dependent upon the relative growth rate of the tree, age and condition of foliage, and the size and stage of development of the nut crop.

Starch was the most variable of the constituents determined, and its concentration appeared to have a marked influence on fruiting.

The rapid disappearance of starch during the spring growth and blossoming period and the decrease of starch and hemicellulose during the nut-filling period show that both growth and fruiting in the pecan are exhaustive processes.

Reducing sugars were low and showed no consistent seasonal variations, thus indicating they are labile forms of carbohydrates. The nonreducing sugars, however, were present in appreciable quantities and varied with the starch although to a lesser extent. These may serve as storage carbohydrates or may be labile forms whose concentration depends largely on that of starch.

The total nitrogen content in the roots was low at all times, but it decreased during rapid spring growth and increased during winter when the trees were dormant and soil conditions were favorable for nitrogen absorption. Nitrogen concentration was largely independent of dry-matter content.

LITERATURE CITED

(1) Association of Official Agricultural Chemists.
1925. official and tentative methods of analysis. Compiled by the committee on editing methods of analysis. Ed. 2, 535 pp., illus. Washington, D. C.

(2) Butler, O. R., Smith, T. O., and Curry, B. E.
1917. physiology of the apple. distribution of food materials in the apple at different periods of vegetation. N. H. Agr. Expt. Sta. Tech. Bull. 13, 21 pp., illus.

(3) CAMERON, S. H.
1933. STARCH IN THE YOUNG ORANGE TREE. Amer. Soc. Hort. Sci. Proc. (1932) 29: 110–114.
(4) CRANE, H. L., and HARDY, MAX B.
1934. INTERRELATION BETWEEN CULTURAL TREATMENT OF PECAN TREES, THE SIZE AND DEGREE OF FILLING OF THE NUTS, AND THE COMPOSITION OF KERNELS. Jour. Agr. Research 49: 643–661.
(5) ——— HARDY, MAX B., LOOMIS, N. H., and DODGE, F. N.
1935. EFFECT OF NUT THINNING ON SIZE, DEGREE OF FILLING, AND ANNUAL YIELD OF PECANS. Amer. Soc. Hort. Sci. Proc. (1934) 31 (v. 32): 29–32.
(6) DAVIS, LUTHER D.
1931. SOME CARBOHYDRATE AND NITROGEN CONSTITUENTS OF ALTERNATE-BEARING SUGAR PRUNES ASSOCIATED WITH FRUIT BUD FORMATION. Hilgardia 5: 119–154, illus.
(7) FINCH, A. H.
1935. FURTHER NOTES ON PECAN FILLING AND MATURITY. Amer. Soc. Hort. Sci. Proc. (1934) 31 (v. 32): 24–28, illus.
(8) ———
1936. ANOTHER SEASON'S STUDIES ON PECAN FILLING AND MATURITY. Amer. Soc. Hort. Sci. Proc. (1935) 32 (v. 33): 41.
(9) ——— and VAN HORN, C. W.
1936. THE PHYSIOLOGY AND CONTROL OF PECAN NUT FILLING AND MATURITY Ariz. Agr. Expt. Sta. Tech. Bul. 62, pp. 421–472, illus.
(10) HEINICKE, A. J., and HOFFMAN, M. B.
1933. THE RATE OF PHOTOSYNTHESIS OF APPLE LEAVES UNDER NATURAL CONDITIONS. I. N. Y. (Cornell) Agr. Expt. Sta. Bull. 577, 32 pp., illus.
(11) HOOKER, H. D., JR.
1920. SEASONAL CHANGES IN THE CHEMICAL COMPOSITION OF APPLE SPURS. Mo. Agr. Expt. Sta. Research Bull. 40, 51 pp., illus.
(12) LECLERC DU SABLON, M.
1904–6. RECHERCHES PHYSIOLOGIQUES SUR LES MATIÈRES DE RÉSERVES DES ARBRES. Rev. Gén. Bot. 16: [341]–368, illus., 1904; 18: 5–25, 1906.
(13) LINK, K. P., and SCHULZ, E. R.
1924. EFFECTS OF THE METHOD OF DESICCATION ON THE NITROGENOUS CONSTITUENTS OF PLANT TISSUES. Jour. Amer. Chem. Soc. 46: 2044–2050.
(14) MURNEEK, A. E.
1928. NITROGEN AND CARBOHYDRATE DISTRIBUTION IN ORGANS OF BEARING APPLE SPURS. Mo. Agr. Expt. Sta. Research Bull. 119, 50 pp., illus.
(15) ———
1929. HEMICELLULOSE AS A STORAGE CARBOHYDRATE IN WOODY PLANTS, WITH SPECIAL REFERENCE TO THE APPLE. Plant Physiol. 4: 251–264, illus.
(16) ———
1934. CARBOHYDRATE STORAGE IN APPLE TREES. Amer. Soc. Hort. Sci. Proc. (1933) 30: 319–321.
(17) ——— and LOGAN, J. C.
1932. AUTUMNAL MIGRATION OF NITROGEN AND CARBOHYDRATES IN THE APPLE TREE WITH SPECIAL REFERENCE TO LEAVES. Mo. Agr. Expt. Sta. Research Bull. 171, 30 pp., illus.
(18) SCHELLENBERG, H. C.
1905. ÜBER HEMICELLULOSEN ALS RESERVESTOFFE BEI UNSEREN WALDBÄUMEN. Ber. Deut. Bot. Gesell. 23: 36–45, illus.
(19) SITTON, B. G.
1931. A PRELIMINARY REPORT ON THE RELATION OF FOLIAGE TO FILLING OF THE PECAN. Natl. Pecan Assoc. Proc. 30: 120–125.
(20) THOR, CLIFFORD J. B., and SMITH, CHARLES L.
1935. A PHYSIOLOGICAL STUDY OF SEASONAL CHANGES IN THE COMPOSITION OF THE PECAN DURING FRUIT DEVELOPMENT. Jour. Agr. Research 50: 97–121, illus.
(21) TOTTINGHAM, W. E., ROBERTS, R. H., and LEPKOVSKY, S.
1921. HEMICELLULOSE OF APPLE WOOD. Jour. Biol. Chem. 45: 407–414, illus.

APPLICABILITY OF NUTRIENT-SOLUTION PURIFICATION TO THE STUDY OF TRACE-ELEMENT REQUIREMENTS OF RHIZOBIUM AND AZOTOBACTER [1]

By ROBERT A. STEINBERG

Associate physiologist, Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, United States Department of Agriculture [2]

INTRODUCTION

Though almost 20 years have elapsed since the introduction by the writer of the method of nutrient-solution purification for studying trace-element requirements of plants, no application of this procedure to studies with bacteria has as yet been made. For reasons readily apparent, it has long seemed desirable to the writer to obtain data affording a direct comparison of the effects of this technique on bacteria and on *Aspergillus niger* Van Tiegh. (*14*).[3] A direct comparison involves the use of the same equipment, chemicals, and technique by the same investigator, so far as possible. The organisms selected for this comparison were *Rhizobium trifolii* Dangeard and *Azotobacter chroococcum* Beij.

The procedures employed necessitated, as usual, the ascertainment of the minimum quantities of the usual ash constituents required for maximum growth, together with the identification and study of the necessary trace elements. Again, it was found essential to devote some attention to the accessory growth substance required by rhizobium in order to limit the extent to which any mineral impurity it might contain would alter or vitiate the results on mineral nutrition.

Though these experiments are, in a manner, incomplete, it should be recalled that their primary objective was a study of the applicability of the method of nutrient-solution purification to bacterial studies. It was considered undesirable, therefore, to go more deeply into the study of these organisms than was necessary for a general comparison with aspergillus. To have done so, even for the purpose of obtaining improved results with the trace elements, would have necessitated changes in equipment, to provide adequate aeration for azotobacter, besides considerable additional work on purification of the accessory growth bodies required by rhizobium.

REVIEW OF LITERATURE

No attempt will be made to present a general summary of the literature, in view of the many excellent reviews that are available. That of Wilson (*18*) on rhizobium and of Burk (*4*) on azotobacter are among the most recent.

[1] Received for publication June 4, 1938; issued September 1938.

[2] The writer expresses his appreciation of the help extended him by various investigators in the U. S. Department of Agriculture. L. A. Burkey, of the Bureau of Dairy Industry, made the microscopic bacterial counts mentioned in this paper, and Daniel Ready, of the Division of Soil Microbiology, Bureau of Plant Industry, the nitrogen determinations. Acknowledgment is further made of cultures and helpful information received from L. T. Leonard and N. R. Smith, also of the latter Division.

[3] Italic numbers in parentheses refer to Literature Cited, p. 476.

Rhizobium and azotobacter are known to require, besides a source of energy, nitrogen, potassium, phosphorus, magnesium, sulphur, iron, calcium, and molybdenum. Various claims also have been made regarding the necessity for other elements, including copper, zinc, manganese, tungsten, silicon, vanadium, and strontium. Furthermore, complete agreement has not as yet been reached regarding the essentiality of an accessory growth factor for rhizobium (*17*). On the other hand, data have begun to accumulate that would indicate that, like the vitamin requirements of animals, the accessory growth-substance requirements of all bacteria and fungi are limited to a circumscribed number of organic compounds the need for which is general and not specific to a single organism and the functions of which in plants are quite analogous to those of vitamins in animals.

METHODS

Rhizobium trifolii Dangeard (E. B. Fred No. 205) and *Azotobacter chroococcum* Beij. (N. R. Smith No. 7) were grown for 4 days at 28° C. in 50-cc portions of a sucrose nutrient solution in 125-cc pyrex Erlenmeyer flasks. Stock cultures of *R. trifolii* were maintained on an agar medium containing sucrose, bactopeptone, malt, and yeast extract, while those of *A. chroococcum* were grown on Ashby agar. Three drops of a freshly prepared bacterial suspension was used to inoculate each flask. Sucrose containing not over 0.0025 percent of ash, reagent chemicals, and water redistilled in pyrex glass were used in the preparation of the nutrient solutions. The flasks were sterilized at 15 pounds' pressure for 30 minutes.

Purification (*14*) with magnesium carbonate was accomplished by heating at 100° C. in the Arnold sterilizer for 20 minutes (10 minutes being allowed for prewarming); and with calcium carbonate, by heating for the same length of time at 15 pounds' pressure in the autoclave. The quantities of carbonate differed in different experiments, the details of which are given later. The nutrient solutions were treated before addition of the trace elements and the organic compounds to be tested. Filtration was through a Jena glass filter of No. 4 porosity.

Each experiment tabulated was the final test of a series performed to determine the optimum concentrations of the individual constituents. The experiments tabulated are typical tests, comprising only 5 to 10 percent of those performed.

Cultures were usually prepared in duplicate and were quite uniform as a rule. The references to the differences in yields of the controls in different experiments do not apply to the data of the same experiment. Reproducibility and consistency of relative results in repeated trials are emphasized rather than the absolute results of an individual experiment.

A Bausch & Lomb nephelometer, provided with 50-mm cups, was used to measure growth as turbidity. Use of a fluctuating turbidity standard, namely, control cultures, proved quite unsatisfactory, since the controls themselves remained unknowns, but this defect was remedied by improvising a turbidity standard consisting of a rod of turbid acrylate condensate. The turbidity of all cultures was measured with the cup at 10 mm. Each millimeter of the turbidity standard was assumed to be equivalent to approximately 10,000,000

rhizobium cells per cubic centimeter or approximately 2,000,000 azotobacter cells per cubic centimeter. The estimates were based on readings of 49.2 mm with a rhizobium suspension of 500,000,000 bacteria per cubic centimeter and 11.3 mm with an azotobacter suspension of 22,320,000 bacteria per cubic centimeter, on the assumption of an exact proportionality between growth and turbidity. A freshly prepared solution containing 25 mg of magnesium sulphate ($MgSO_4.7H_2O$) plus 50 mg of potassium phosphate (K_3PO_4) per 100 cc gave a reading of 25.2 mm with the solution cup set at 10.0 mm. Acidities were determined with a Leeds and Northrup quinhydrone electrode.

The values in table 1 are of interest in view of the scarcity of such data and the increasing use of the nephelometer for measuring bacterial growth as turbidity. They are the results of several different experiments.

Though these observations are limited in both number and range covered, they would seem to indicate a change in proportionality between bacterial count and turbidity depending upon the extent of growth. The average value of the ratio between bacterial count and turbidity is 2,009,000 for readings between 2.0 and 11.3 mm. An increased precision should be readily attainable through the use of a more carefully calibrated, uniform turbidity standard, manipulation to obtain numerical readings of greater magnitude, and mechanical means to form a uniform suspension of bacteria. Also, the use of a colloid to stabilize the suspension may be advisable. The rapidity with which readings may be made would warrant further investigation of the possibilities of the nephelometer.

TABLE 1.—*Comparison of growth measurements of azotobacter as determined by turbidity readings and by microscopic bacterial counts*

Turbidity (millimeters)	Bacteria	Ratio of number of bacteria to turbidity	Turbidity (millimeters)	Bacteria	Ratio of number of bacteria to turbidity	Turbidity (millimeters)	Bacteria	Ratio of number of bacteria to turbidity
	Millions per cubic centimeter	*Millions per millimeter*		*Millions per cubic centimeter*	*Millions per millimeter*		*Millions per cubic centimeter*	*Millions per millimeter*
0.5	5.336	10.672	2.5 (−Fe)	2.828	1.131	3.8	7.360	1.937
1.0	8.372	8.372	2.5 (−Mo)	4.692	1.877	4.5	13.104	2.912
1.7	7.304	4.297	2.8	8.677	3.099	11.3	22.320	1.975
2.0	2.376	1.188	3.7	6.120	1.654			

EXPERIMENTAL RESULTS WITH RHIZOBIUM

After considerable effort to grow rhizobium in solutions free from organic matter other than sucrose, further attempts were abandoned and Difco bactopeptone was adopted as a source of growth factor. It was found that yields of only 1 to 2 mm (2 to 4 million bacteria per cubic centimeter) could be obtained without the addition of organic material containing an accessory growth factor, whereas yields corresponding to 45 mm or more could be obtained by its addition. Repeated tests with salts of 77 of the chemical elements at different concentrations gave no indication to substantiate the belief that the poor growth without growth factor was due to any single inorganic deficiency. The quantity of bactopeptone to be employed was set at

50 mg per liter, i. e., 5 parts per 100,000. This is a somewhat suboptimal quantity, but it was adopted because of the turbidity characteristics of the cultures at the time of harvest after 4 days of growth.

The results given in experiment 5 (table 2) are typical of those obtained with an unpurified solution containing 50 mg of bactopeptone per liter, except that the yield of the control varied somewhat from experiment to experiment. The nonaddition of iron or of molybdenum to the nutrient solution did not result in a decrease in growth, while a slight decrease took place through withholding manganese or calcium. It is interesting to compare these findings with those of experiment 7 (table 2), in which a partially purified bactopeptone preparation in absolute alcohol was used. The use of this preparation, of which more will be said later, evidently had decreased the content of mineral impurities in the bactopeptone since a decrease in the percentage of maximum yield followed the omission of any one of the trace elements. Purification of the nutrient solution with magnesium carbonate seemed effective to the same degree, as reference to experiment 6 shows. The best results, however, were obtained by combining purification of the nutrient solution with the use of purified bactopeptone, as indicated in experiment 8.

Many additional experiments, other than those tabulated, led to the same conclusions: Omission of iron, manganese, molybdenum, or calcium from the nutrient solution gave decreased growth of rhizobium. No evidence, however, could be obtained for the essentiality of any of the other elements tested.

Experiment 3 (table 2) shows the effect of substituting sodium nitrate ($NaNO_3$) for ammonium nitrate (NH_4NO_3) in the nutrient solution, and experiment 2 the result of the omission of inorganic nitrogen. The solution in the latter experiment contained 13 mg of nitrogen per liter, owing to the addition of bactopeptone. Under these conditions, no fixation of nitrogen occurred in the control.

TABLE 2.—*Effects of trace elements on the growth of Rhizobium trifolii in different solutions for 4 days at 28° C.*

Trace element omitted	Effects with solution [1] used in—																							
	Experiment 1 (sucrose, 20.00; NH_4NO_3, 0.15; K_2HPO_4, 0.15; $MgSO_4.7H_2O$, 0.09; bactopeptone, 0.05; $4MgCO_3.Mg(OH)_2.5H_2O$, 0.40 g per liter, filtered hot)			Experiment 2 (sucrose,[2] 10.00; K_2HPO_4, 0.15; $MgSO_4.7H_2O$, 0.09; bactopeptone, 0.05; $4MgCO_3.Mg(OH)_2.5H_2O$, 0.50 g per liter, filtered hot)			Experiment 3 (sucrose, 10.00; $NaNO_3$, 0.32; K_2HPO_4, 0.15; $MgSO_4.7H_2O$, 0.09; bactopeptone, 0.05; $4MgCO_3.Mg(OH)_2.5H_2O$, 0.50 g per liter filtered hot)			Experiment 4 (sucrose, 5.00; NH_4NO_3, 0.15; K_2HPO_4, 0.15; $MgSO_4.7H_2O$, 0.09; bactopeptone, 0.05; $4MgCO_3.Mg(OH)_2.5H_2O$, 0.50 g per liter, filtered hot)			Experiment 5 (sucrose, 5.00; NH_4NO_3, 0.25; K_2HPO_4, 0.08; $MgSO_4.7H_2O$, 0.04; bactopeptone, 0.05 g per liter)			Experiment 6 (sucrose, 3.00; NH_4NO_3, 0.15; K_2HPO_4, 0.15; $MgSO_4.7H_2O$, 0.09; bactopeptone, 0.05; $4MgCO_3.Mg(OH)_2.5H_2O$, 0.50 g per liter, filtered hot)			Experiment 7 (sucrose, 3.00; NH_4NO_3, 0.25; K_2HPO_4, 0.08; $MgSO_4.7H_2O$, 0.04 g per liter; bactopeptone, 10 cc)			Experiment 8 (sucrose, 3.00; NH_4NO_3, 0.20; K_2HPO_4, 0.15; $MgSO_4.7H_2O$, 0.07; $4MgCO_3.Mg(OH)_2.5H_2O$, 0.50 g per liter; bactopeptone, 10 cc. Filtered hot)		
	Yield [3]	Proportion of maximum yield	Acidity at harvest	Yield [3]	Proportion of maximum yield	Acidity at harvest	Yield [3]	Proportion of maximum yield	Acidity at harvest	Yield [3]	Proportion of maximum yield	Acidity at harvest	Yield [3]	Proportion of maximum yield	Acidity at harvest	Yield [3]	Proportion of maximum yield	Acidity at harvest	Yield [3]	Proportion of maximum yield	Acidity at harvest	Yield [3]	Proportion of maximum yield	Acidity at harvest
	Mm	*Pct.*	*pH*	*Mm*	*Pct.*	*pH*	*Mm*	*Pct.*	*pH*	*Mm*	*Pct.*	*pH*	*Mm*	*Pct.*	*pH*	*Mm*	*Pct.*	*pH*	*Mm*	*Pct.*	*pH*	*Mm*	*Pct.*	*pH*
Fe	9.1	26.2	7.37	7.6	73.8	7.27	24.3	66.4	6.80	12.0	43.0	7.38	18.2	148.0	5.03	10.2	52.6	7.17	11.7	80.1	4.89	7.4	31.1	7.05
Mn							34.3	93.7	6.66	25.8	92.5	7.04	11.3	91.9	4.92	17.2	88.7	7.15	8.2	56.2	4.58	14.4	60.5	7.03
Mo	35.8	102.9	6.74	9.9	96.1	7.02	30.8	94.2	6.59	25.5	91.4	6.95	12.9	104.9	4.78	19.2	99.0	7.06	12.3	84.3	4.55	20.8	87.4	6.97
Ca	31.9	91.7	6.59	8.4	81.6	7.02	29.1	79.5	6.64	21.7	77.8	7.07	10.9	88.6	4.78	15.7	80.9	7.18	12.4	84.9	4.55	12.9	54.2	7.07
None	34.8	100.0	6.54	10.3	100.0	6.81	36.6	100.0	6.39	27.9	100.0	6.88	12.3	100.0	5.18	19.4	100.0	6.64	14.6	100.0	4.84	23.8	100.0	6.82
Maximum [4]	39.4			12.7			40.2			28.6			19.2			21.8			15.2			29.4		
pH [5]			8.00			7.75			7.30			7.80			6.79			8.26			7.17			8.33

[1] Iron, manganese, molybdenum, and calcium were added to the extent of 0.06, 0.01, 0.01, and 0.30 mg per liter, respectively. All constituents of experiment 1 were in minimum concentration for maximum growth with 20-percent sucrose.

[2] This solution contained 13 mg N per liter initially and at harvest.

[3] The extent of growth was estimated by the turbidity of the culture. The values give the depths of the fixed turbidity standard required to match a 10-mm depth of the culture.

[4] Maximum individual yield obtained in the experiment.

[5] Initial acidity of the nutrient solution.

A comparison of the effectiveness of some organic substances and of their extracts in aiding the growth of rhizobium is given in table 3, since these results influenced the choice of bactopeptone as a source of growth factor. The results are stated as percentage increase in yield at a concentration of 1.0 mg per liter. Bactopeptone and yeast extract were most effective and to about the same degree. The difference in effectivity of these nitrogen-rich sources indicated that organic nitrogen as such was not the factor underlying these variations. Attempts to obtain the effective substance in purer form by simple extraction with solvents were unsuccessful. Nor was sublimation of bactopeptone at 147° C. and 5- to 10-mm pressure more successful. Though considerable material sublimed, its effectiveness was lower (3-percent increase at 1 mg per liter) than the original substance. Sublimation of a bactopeptone preparation obtained by precipitation with lead, adsorption with charcoal, and extraction with ammoniacal acetone seemed more efficacious, since a growth increase of 39.2 percent with approximately 1.0 mg per liter of substance was obtained in one trial.

Miscellaneous additional tests were made in the attempt to obtain a purer product. With 1.0 g of bactopeptone dissolved in 100 cc of

TABLE 3.—*Influence of organic substances on the growth of Rhizobium trifolii for 4 days at 28° C. in a nutrient solution purified with magnesium carbonate*

Organic substance added		Growth measured nephelometrically	Computed [1] increase in yield for 1 mg substance per liter
Material and method of production [2]	Quantity per liter		
	Milligrams	*Millimeters*	*Percent*
Control		1.2	
Bactopeptone, Difco	50.0	44.3	72.0
Extracted cold with 95-percent alcohol	13.0	14.9	87.7
Extracted cold with absolute alcohol	6.0	4.3	43.3
Extracted cold with acetone	.8	1.7	52.5
Extracted hot with 95-percent alcohol	23.6	18.9	62.3
Extracted hot with acetone	5.4	4.8	55.6
Nutrose, Pfanstiehl [3]	50.0	4.3	5.2
Extracted cold with 95-percent alcohol	3.6	3.4	41.7
Extracted cold with absolute alcohol	3.2	2.1	56.2
Extracted cold with acetone	2.2	1.6	60.0
Peptone, Witte	50.0	3.5	5.8
Yeast extract, Difco	50.0	53.9	88.0
Malt extract, Difco	50.0	23.5	37.2

[1] The assumption of exact proportionality is probably true only for the lower values (*10*), nor are corrections made for ash content.

[2] 1 gram of the solid was shaken with 100 cc of liquid and the clear supernatant solution tested. Hot extraction was with a Soxhlet extractor, the extract from 10 g being adjusted to 100 cc.

[3] In a previous experiment with an unpurified solution the yield of the control was equivalent to 1.3 mm; with 50 mg bactopeptone per liter it was 20.9 mm; and with 50 mg nutrose per liter it was 30.4 mm.

0.2 N sulphuric acid (H_2SO_4), it was found that growth factor was not destroyed by continued heating on the water bath. Neither was it precipitated by an excess of barium carbonate nor by phosphotungstic acid. The bactopeptone control, at 50 mg per liter, gave a growth value of 34.8 mm; the unheated control gave 35.2 mm; while that subjected to heating gave 34.2 mm. With barium carbonate ($BaCO_3$) the precipitated material gave a growth increase of 10.3 percent as compared to an increase of 56.1 percent in the unprecipitated material. With phosphotungstic acid, the growth increase in the

precipitated material was 9.6 percent and that in the filtrate 40.5 percent.

Treatment of a neutral solution of bactopeptone with basic lead acetate followed by deleading with potassium bicarbonate ($HKCO_3$) did not decrease the growth-factor content, as also reported by Clark, (7). If the solution was then treated with Merck's "Medicinal Charcoal, extracted with acid," the factor was adsorbed. Elution of the adsorbed factor was accomplished with ammoniacal acetone (1+9) or ammoniacal absolute alcohol (1+9). The latter gave a deep brownish-red solution and was the method by which the bactopeptone preparation used in some of the experiments of table 2 was prepared. This preparation could be freed of color with little loss in activity by diluting the neutral solution with water and extracting with amylic alcohol. The increase in effectiveness of the organic material by these methods was slight. The acetone extraction material, for example, gave a growth increase of 137 percent at the 1.0-mg level, as compared to an increase of 72 percent with the original substance.

Nutrose treated by the same procedures gave an increase in yield of 66 percent at the 1.0 mg per liter level, which was about equal to that obtained by simple extraction with absolute alcohol or acetone. Mild hydrolysis of nutrose did not lead to an increase in effectiveness.

Finally, the compounds in the following list were tested for their ability to bring about growth increases, but unsuccessfully. Lecithin (from eggs) sometimes brought about slight increases, the others practically none at all. Occasionally it was thought that one of these compounds was effective, but a careful check failed to verify this interpretation.

Glycerol
Raffinose
Trehalose
Maltose
Galactose
d-Mannose
d-Mannitol
Sodium glycerophosphate
Dextrose
Levulose
i-Inositol
Lactose
Malic acid
Asparagine
Malonic acid
Taurine
Cholesterol
Creatine
β-Carotene
Choline hydrochloride
β-Alanine
Tannic acid
β-Indolylpropionic acid
Glucosamine hydrochloride
Vitamin B_1
Vitamin C
Lactoflavine
Sodium magnesium chlorophyllin
Sodium iron chlorophyllin
Adenine
Uracil
Guanine
Hydroxylamine hydrochloride
Hydrazine hydrochloride
l-Tyrosine
dl-Valine
α-Alanine, racemic
d-Arginine
l-Aspartic acid
l-Cystine
d-Glutamic acid
Glycine
l-Histidine dihydrochloride
l-Hydroxyproline
l-Leucine
dl-Isoleucine
d-Lysine dihydrochloride
dl-Methionine
α-Phenylalanine
β-Phenylalanine
l-Proline
l-Tryptophane
Glycogen
dl-Serine
dl-Norleucine
Uric acid
Sodium nucleinate
Xanthine
Pyruvic acid
Glycolic acid
Thiourea
Allantoin
Lecithin (egg)

One interesting point had been encountered in these experiments on the purification of the accessory factor required by rhizobium; namely, that nutrose, when added to an unpurified nutrient solution, was as effective as bactopeptone in bringing about increased growth; whereas, when added to a solution purified with magnesium carbon-

ate [4] it was totally ineffective. The uninoculated control with the purified solution containing 50 mg of nutrose per liter usually had a value of 1 to 2 mm, while the inoculated control with nutrose had a value of 3 to 4 mm. The control without nutrose gave no growth. Evidently the magnesium carbonate treatment removed from the nutrient solution some factor, essential for growth of rhizobium, in which nutrose is deficient. This deficiency could not be supplied by subsequent addition of nitrogen, phosphorus, etc. Repeated tests with nutrose and salts of the chemical elements at concentrations of 50 to 500 parts per billion failed to identify the deficiency. The substances listed on page 465 were then tested in combination with nutrose and proved uniformly ineffective, with the exception of lecithin, which was the last one tried. One milligram of lecithin per liter in a purified solution containing 50 mg of nutrose per liter increased the growth of rhizobium from 2.7 mm to 39.4 mm, after correction for the turbidity caused by the nutrose. The need for the joint presence of nutrose and lecithin indicated that the presence of two accessories is required by rhizobium.

The possibility that the milligram of lecithin per liter added to the solution in conjunction with nutrose served as a means of replenishing a deficiency of nitrogen, phosphorus, etc., is quite remote. The quantity involved is too small. Nutrose itself contains nitrogen, phosphorus, and many inorganic impurities. Moreover, other organic compounds containing nitrogen and phosphorus could not overcome the deficiency. Lastly, and most important, is the fact that none of the solutions of experiments tabulated in this paper was deficient in phosphate, as ascertained by repeated tests.

The almost complete removal from the nutrient solution of the trace of an accessory factor, presumably introduced with the sucrose, by magnesium carbonate was duplicated with bactopeptone and barium carbonate ($BaCO_3$). To 2.5 mg of bactopeptone, dissolved in 5 cc of 0.2 N sulphuric acid, an excess of barium carbonate was added; the residue, after filtration, was extracted with ammoniacal absolute alcohol (1+9); and the solvent was evaporated. The uninoculated control in one experiment gave a value of 1.8 mm for the turbidity due to nutrose; the filtrate from the barium carbonate residue gave 2.2 mm; and the residue from the ammoniacal alcohol gave 21.0 mm. This procedure, therefore, was effective at a concentration of 0.5 mg bactopeptone per cubic centimeter in removing one or both accessories almost completely (about 98 percent) from the solution, but ineffective at a concentration of 10 mg of bactopeptone per cubic centimeter. No tests were performed to confirm the necessity for using an acid (or sulphate ion ?), instead of a neutral solution, for the precipitation. It should be noted in this connection that purification of the nutrient solution with magnesium carbonate removed the impurity of the sucrose almost completely, whereas the quantity removed when 50 mg of bactopeptone per liter was present made little perceptible difference in growth. Apparently barium carbonate was more effective than magnesium carbonate, if it is assumed that the presence of other constituents is immaterial.

[4] The composition of the purified nutrient solution was as follows: Redistilled water, 1,000 cc; sucrose, 20.0 g; NH_4NO_3, 0.30 g; K_2HPO_4, 0.12 g; $MgSO_4.7H_2O$, 0.06 g; and $4MgCO_3.Mg(OH)_2.5H_2O$, 0.10 g, filtered hot. To the clear solution was added nutrose, iron, molybdenum, and calcium, in quantities of 50.0, 0.05, 0.01, and 0.30 mg per liter, respectively. Its acidity was pH 7.5 to 7.8.

Tests finally were made with some of the growth accessories identified as necessary for certain micro-organisms. These were i-inositol, required by yeasts; pimelic and nicotinic acids, by the diphtheria bacillus (*12*); and vitamin B_1 by *Phycomyces* and other organisms (*6, 13*). *Staphylococcus* has also been found by Knight (*9*) to require nicotinic acid and vitamin B_1. Nutrose and lecithin, too, were included. They were tested singly and in pairs at 1 to 2 mg per liter but proved uniformly ineffective, except nutrose plus lecithin at 50 and 1 mg per liter, respectively.

As a consequence of the unsuccessful search for an accessory growth factor in relatively pure form the use of bactopeptone was adopted as standard. The use of alcoholic solutions of bactopeptone was found desirable to prevent bacterial decomposition and to decrease the ash content. Some decomposition of the accessory growth bodies seems to occur in alcoholic solution, however.

Little has appeared in the literature (*16*) on the necessity for a careful check of the various claims for the existence of growth factors, as well as of the presence of trace elements in the organic materials used to culture micro-organisms. The following tabulation should, therefore, prove of value as a measure of the extent to which organic substances, some of which are in constant use for the preparation of culture media, are contaminated with inorganic materials.

Contaminants found spectroscopically[1] *in some of the materials used in growth studies with rhizobium*

Material:	Elements identified spectroscopically[2]
Bactopeptone, Difco	Na, Ca, Mg, K, Al, Sr, Fe, P, Mn, Cu, Pb, Si, Ba(?), Li.
Peptone, Witte	Na*, Ca*, Mg*, Sr*, Fe*, Si*, B, Mn, K, Cu, Al, Sn, Pb, V(?), Ba.
Malt extract, Difco	Ca*, Mg*, Si, Cu, Mn, Fe, Al, P, K, Na, Ba, Sn, Pb, V(?), Ti(?), Sr.
Yeast extract, Difco	Fe*, Ca*, Mg*, Sr*, Na*, K*, Ba, Mn, Cu, Al, Pb, V(?), Si.
i-Inositol, Pfanstiehl	Ca*, Mg*, Fe*, Pb, Cu, Si, Al, V(?), Mn.
Cholesterol, Pfanstiehl	Cu, Mg(?).
Sodium magnesium chlorophyllin.	Ca*, Fe*, Sr, Cu, Pb, Al, V, Sn(?), Na, Mg, Si, Cd, Pt, Zn, Mn.
Casein, Pfanstiehl	P, Mg, Cu, Pb, Ag, Na, Zn(?).

[1] These data were obtained by B. C. Brunstetter, associate biochemist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, with a Bausch & Lomb large-size quartz spectograph and carbon arc.

[2] Strong traces are indicated by asterisks (*); doubtful, by question marks.

EXPERIMENTAL RESULTS WITH AZOTOBACTER

Cultural work with azotobacter also was handicapped by the inability to duplicate growth values for the controls in successive experiments despite care in the preparation of the nutrient solution, including the use of stock solutions of the inorganic constituents. The results with trace elements shown in table 4 have been duplicated frequently, however, and there can be little doubt, therefore, of their reliability. Azotobacter, like rhizobium, gave decreased yields when traces of iron, manganese, molybdenum, or calcium were omitted from nutrient solutions with both fixed and free nitrogen. This was evident even in the unpurified solution (experiment 1), and was plainer, at least for iron and calcium, with the purified solutions of experiments 2 and 3 (table 4).

TABLE 4.—*Effects of trace elements on growth of Azotobacter chroococcum in different solutions for 4 days at 28° C.*

Trace element omitted	Effect with solution [1] used in—																							
	Experiment 1 (sucrose, 20.00; K_3PO_4, 0.18; $MgSO_4.7H_2O$, 0.09 g per liter)			Experiment 2 (sucrose, 20.00; K_3PO_4, 0.25; $MgSO_4.7H_2O$, 0.15; $4MgCO_3.Mg(OH)_2.5H_2O$, 0.10 g per liter, filtered hot)			Experiment 3 (sucrose, 20.00; K_3PO_4, 0.18; $MgSO_4.7H_2O$, 0.09; $CaCO_3$, 0.30 g per liter, filtered hot)			Experiment 4 (sucrose, 5.00, K_3PO_4, 0.18; $MgSO_4.7H_2O$, 0.09; NaCl, 0.20 g per liter)			Experiment 5 (sucrose, 5.00; $NaNO_3$, 0.18; K_3PO_4, 0.18; $MgSO_4.7H_2O$, 0.09; NaCl, 0.20 g per liter)			Experiment 6 (sucrose, 5.00; K_3PO_4, 0.09; $MgSO_4.7H_2O$, 0.09; $NaHCO_3$, 0.10 g per liter)			Experiment 7 (sucrose, 3.00; $NaNO_3$, 0.20; K_3PO_4, 0.25; $MgSO_4.7H_2O$, 0.20; NaCl, 0.20; $4MgCO_3.Mg(OH)_2.5H_2O$, 0.30 g per liter, filtered hot)			Experiment 8 (sucrose, 3.00; $NaNO_3$, 0.20; K_3PO_4, 0.25; $MgSO_4.7H_2O$, 0.20; NaCl, 0.20; $CaCO_3$, 1.00 g per liter, filtered hot)		
	Yield [2]	Proportion of maximum yield	Acidity at harvest	Yield [2]	Proportion of maximum yield	Acidity at harvest	Yield [2]	Proportion of maximum yield	Acidity at harvest	Yield [2]	Proportion of maximum yield	Acidity at harvest	Yield [2]	Proportion of maximum yield	Acidity at harvest	Yield [2]	Proportion of maximum yield	Acidity at harvest	Yield [2]	Proportion of maximum yield	Acidity at harvest	Yield [2]	Proportion of maximum yield	Acidity at harvest
	Mm	*Pct.*	*pH*	*Mm*	*Pct.*	*pH*	*Mm*	*Pct.*	*pH*	*Mm*	*Pct.*	*pH*	*Mm*	*Pct.*	*pH*	*Mm*	*Pct.*	*pH*	*Mm*	*Pct.*	*pH*	*Mm*	*Pct.*	*pH*
Fe	5.1	67.1	6.85	1.7	58.6	6.87	0.3	18.8	7.06	4.7	75.8	7.02	4.5	40.0	7.02	2.0	38.5	7.25	2.5	36.2	6.97	2.8	28.0	6.93
Mn	7.4	97.4	6.84	3.4	117.2	6.84	2.0	125.0	6.85	6.5	104.8	6.86	9.5	84.1	7.12	4.8	92.3	7.21	6.2	90.0	7.05	6.0	60.0	7.03
Mo	4.8	63.2	6.94	2.0	69.0	6.87	1.5	93.8	6.87	6.3	101.6	6.87	10.0	88.5	7.03	3.9	75.0	7.16	8.3	123.0	7.07	6.8	68.0	7.04
Ca	5.7	75.0	6.94	.5	17.2	7.26	.6	37.5	7.19	0	0	7.49	9.3	82.3	7.09	0	0	7.60	0	0	7.37	0	0	7.13
None	7.6	100.0	6.72	2.9	100.0	6.81	1.6	100.0	6.86	6.2	100.0	6.77	11.3	100.0	6.96	5.2	100.0	7.15	6.9	100.0	6.77	10.0	100.0	6.99
Maximum [3]	7.6	------	------	4.6	------	------	3.0	------	------	6.9	------	------	13.6	------	------	6.0	------	------	8.4	------	------	10.0	------	------
pH [4]	------	------	7.94	------	------	8.07	------	------	8.32	------	------	8.06	------	------	8.09	------	------	7.22	------	------	7.55	------	------	7.47

[1] Iron, manganese, molybdenum, and calcium were added to the extent of 0.06, 0.03, 0.02, and 0.30 mg per liter, respectively. All constituents were in minimum concentration for maximum growth with 20-percent sucrose.

[2] The extent of growth was estimated by the turbidity of the culture. The values give the depths of the fixed turbidity standard required to match a 10-mm depth of the culture.

[3] Maximum individual yield obtained in the experiment.

[4] Initial acidity of the nutrient solution.

The growth with fixed nitrogen in experiment 5 (table 4) was almost double that obtained with free nitrogen. Moreover, the presence of fixed nitrogen as sodium nitrate ($NaNO_3$) did not eliminate the need for any of the trace elements required for growth with atmospheric nitrogen. Attention is also directed to the effect of sodium ion in experiments 4 to 8 (table 4), on the omission of calcium. The reason for the addition of sodium chloride was that it had been found capable, apparently, of causing increased growth, though this increase later proved to be fallacious. Other tests, moreover, had shown that though potassium phosphate (K_3PO_4) was present in optimum amount it could be halved in quantity without diminishing growth, if the deficit was replaced by potassium or sodium bicarbonate ($HKCO_3$ or $HNaCO_3$). Experiments 7 and 8 (table 4) illustrate the effects of purification with magnesium and calcium carbonate on the removal of trace elements in the presence of fixed nitrogen.

Aeration, it was assumed, was the limiting factor for growth with azotobacter in the preceding experiments. The use of 25 cc, instead of 50 cc, of nutrient solution in the 125-cc flasks, increased the growth of the control in experiment 7 (table 4) from 6.9 mm to 22.0 mm, and in experiment 8 from 10.0 mm to 27.8 mm. It is plainly evident, therefore, that none of the constituents of these solutions were deficient in quantity at the lower aeration level.

The effects of a somewhat higher but still insufficient aeration level are shown in table 5, in which 25 cc of culture solution was used in 200-cc flasks instead of 50 cc in 125-cc flasks as in the preceding experiments. Increased aeration brought about very marked increases in growth and slight increases in the concentrations of mineral constituents required. To judge from the fragmentary data available, optimum aeration for this strain would require the use of 1-liter Erlenmeyer flasks with not over 25 cc of nutrient solution, or a depth of liquid of about 3 mm. This is the depth employed by Kostytschew et al. (*11*), who obtained nitrogen fixation of about 25 mg of nitrogen per gram of sugar used. Here again the increase in growth with improved aeration demonstrated the presence of nutrient constituents in ample quantity for the level used.

The possibility of the complete substitution of calcium by strontium (*5, 8*) was tested in a solution without fixed nitrogen, identical with that of experiment 6 (table 4). It had an initial reaction of pH 7.54 and growth in the control was lower than usual, being only 1.3 mm or about 2,600,000 bacterial cells per cubic centimeter (p. 463). Iron, manganese, molybdenum, and calcium were added, as usual, in concentrations of 0.06, 0.03, 0.02, and 0.30 mg per liter respectively. Omission of calcium resulted in a complete cessation of growth. The addition of strontium as chloride, in steps of 0.05 mg per liter up to a maximum concentration of 0.50 mg per liter, proved incapable of aiding growth in any degree in the calcium-free solution. The strontium salt contained 0.0013 percent of calcium, being practically spectroscopically pure. In other experiments in which only partial removal of calcium was accomplished, strontium, however, could be substituted for the deficiency, at least partially. A duplicate of experiment 1 (table 4) gave a yield of 5.5 mm and percentages of maximum yield of 58.2, 100.0, 103.6, and 25.5, respectively, for iron, manganese, molybdenum, and calcium. The initial reaction was

pH 8.16, and the reaction at time of harvest ranged from pH 6.74 for the control to pH 6.91 for minus calcium. Addition of strontium to the minus-calcium culture in equivalent quantity increased the yield to 72.7 percent of maximum, or 27.3 percent less than that of the control with calcium. Other tests gave about 82 and 70 percent replacement (controls, 7.6 and 5.7 mm). Reagent strontium chloride seemed equivalent to calcium chloride.

TABLE 5.—*Effects of trace elements on growth of Azotobacter chroococcum in different solutions for 4 days at 28° C. when 25 cc of nutrient solution was used in 200-cc flasks*

Trace element omitted	Effect with solutions [1] used in—											
	Experiment 1 (sucrose, 10.0; K_2PO_4, 0.25; $MgSO_4.7H_2O$, 0.09 g per liter)			Experiment 2 (sucrose 10.0; K_2PO_4, 0.09; $MgSO_4.7H_2O$, 0.09; $HNaCO_3$, 0.10 g per liter)			Experiment 3 (sucrose, 10.0; K_2PO_4, 0.25; $MgSO_4.7H_2O$, 0.20; $CaCO_3$, 0.50 g per liter, filtered hot)			Experiment 4 (sucrose, 10.0; $NaNO_3$, 0.20; K_2PO_4, 0.25; $MgSO_4.7H_2O$, 0.20; $CaCO_3$, 0.50 g per liter, filtered hot)		
	Yield [2]	Proportion of maximum yield	Acidity at harvest	Yield [2]	Proportion of maximum yield	Acidity at harvest	Yield [2]	Proportion of maximum yield	Acidity at harvest	Yield [2]	Porportion of maximum yield	Acidity at harvest
	Mm	Pct.	*pH*	*Mm*	*Pct.*	*pH*	*Mm*	*Pct.*	*pH*	*Mm*	*Pct.*	*pH*
Fe	3.8	47.5	7.01	6.5	48.2	7.01	1.2	17.7	6.84	8.8	59.5	6.80
Mn	7.8	97.5	6.82	12.5	92.6	6.77	7.9	116.2	6.55	16.1	108.8	6.92
Mo	6.4	80.0	6.86	9.5	70.4	6.87	9.0	132.4	6.51	20.0	135.1	6.89
Ca	5.5	68.8	6.95	7.7	57.0	6.88	1.7	25.0	6.79	8.5	57.4	6.92
None	8.0	100.0	6.75	13.5	100.0	6.51	6.8	100.0	6.69	14.8	100.0	6.87
Maximum [3]	13.6	------	------	26.1	------	------	14.5	------	------	26.6	------	------
pH [4]	------	------	8.26	------	------	7.64	------	------	8.03	------	------	7.47

[1] Iron, manganese, molybdenum, and calcium were used at concentrations of 0.08, 0.02, 0.02, and 0.40 mg per liter, respectively.
[2] The extent of growth was estimated by the turbidity of the cultures. The values give the depth of the fixed turbidity standard required to match a 10-mm depth of the culture.
[3] Maximum individual yield of control obtained with 500-cc flasks.
[4] Initial acidity of nutrient solution.

Many of the experiments with azotobacter included tests for ammonia (Nessler), hydroxylamine and nitrite (Griess), and nitrate (diphenylamine), which were performed on the nutrient solution without removal of the bacteria (*11*). Hydroxylamine was oxidized with iodine to nitrite. At both levels of aeration tested, the cultures with free nitrogen gave positive tests for ammonia only (*11*), nor did a trace-element deficiency alter this response. With nitrogen supplied as nitrate, the cultures gave tests for all four constituents at the lower aeration level (50 cc nutrient solution in 125-cc flasks). At the higher aeration level (25 cc nutrient solution in 200-cc flasks), the cultures with nitrate nitrogen gave positive tests for ammonia and unconsumed nitrate, and for hydroxylamine when iron, manganese, or calcium was not added to the solution. Only the minus-calcium cultures gave a positive test for nitrite. No tests, unfortunately, could be made at the optimum aeration level, but it would seem quite

probable that at this level also a graduated deficiency of the trace elements may result in the accumulation of sufficient hydroxylamine in the nitrate solution to give a positive reaction.

DISCUSSION

Inability to duplicate closely the yields of controls in successive experiments has had the effect of diminishing the value of the data. This was overcome to some degree by frequent repetition and check. Under these conditions, of course, it was not possible to more than approximate optimum solutions for growth. Though the cause of these uncontrolled variations is unknown, continued tests with many of the chemical elements failed to identify one of these as the cause. Duplicate cultures, however, gave quite uniform results, and the same was often true for different treatments of the same experiment.

The use of the method of nutrient-solution purification with magnesium or calcium carbonate led to some improvement in results, but less than that obtained with aspergillus. The reason for this probably lies partly in the very small increase in mass of bacteria as compared to fungi for the same quantity of nutrient. On the assumption of even a 200-mg yield per 1.0 g of sucrose, the coefficient of utilization would be only 20 as compared to 50 for aspergillus. It is not surprising, therefore, that the quantities of essential impurities remaining in the nutrient solution after purification, which are still partially effective in the case of aspergillus, are even more effective with rhizobium and azotobacter, especially at low growth levels, in supplying the deficiencies of the elements experimentally omitted. This does not mean, however, that the method of nutrient-solution purification is useless with bacteria but rather that an additional improvement in technique is highly desirable, a fact already known from investigations with aspergillus.

Another factor making for poor deficiency results with the trace elements is the low growth of the controls. Increased growth of control is generally accompanied by improved deficiency results, as has been demonstrated with aspergillus by the writer. This may be accomplished to a considerable extent by the use of greater quantities of bactopeptone with rhizobium and improved aeration with azotobacter, without a further increase in mineral ingredients. It is doubtful, however, that all inorganic constituents in the solutions of table 5 will be present in ample amount when growth factor and oxygen are no longer limiting factors for the respective organisms.

Vigor of culture is best expressed as the maximum bacterial count obtained in these experiments. That for rhizobium was 500,000,000 bacteria per cubic centimeter by actual count. The maxima for azotobacter were estimated at 56,000,000 per cubic centimeter with fixed nitrogen, and 19,000,000 without; improved aeration led to increased counts of 52,000,000 per cubic centimeter without fixed nitrogen and 166,000,000 with fixed nitrogen. Marked increase in growth of rhizobium could be brought about by increased bactopeptone. The quantity of nitrogen fixed by a 50-cc culture in a 125-cc flask of a turbidity of 4.5 mm was too little to be determined by microtitration.

Except for the inability to duplicate successive controls to within a small variation, the data on the trace elements are, on the whole, quite similar to those with aspergillus even at the foregoing nutrition

levels. Omission of an essential element led to a decrease in growth and diminished the rise in acidity accompanying growth. The need of these organisms for iron, manganese, molybdenum, and calcium, even under the suboptimum conditions of these experiments, appears to be specific on the basis of many tests with salts of 77 elements. On the basis of the experience gained in these experiments, the belief was developed that the mineral requirements of the bacteria for growth are, in general, as rigid and inflexible as those for fungi and other forms of life, and that the evidence to the contrary is entirely inadequate, being based on results due to use of impure chemicals.

The evidence for the necessity of a growth factor by rhizobium is based partially on the inability to obtain growth in the absence of this factor by the addition of salts of the chemical elements or by the addition of many organic compounds capable of serving as sources of carbon and nitrogen. The degree of dependence on an accessory growth factor would appear to differ with the strain (*1*, *2*). With the trace of accessory growth factor in the sucrose, *Rhizobium trifolii* No. 532 of L. T. Leonard, which was also tested to a considerable extent, invariably gave larger yields than the No. 205 strain of E. B. Fred and also differed from the latter by giving slight increases in growth with sodium thiosulphate. Moreover, with sufficient decrease in accessory-growth-factor content of the nutrient solution, the No. 532 strain is unable to grow. With this strain, also, the need for an accessory growth factor cannot be avoided by the use of mineral constituents or organic carbon and nitrogen compounds. Furthermore, the use of nitrogen as ammonium salt, nitrate, or asparagine did not preclude the need for an accessory growth factor.

The data afford additional confirmation for the essentiality of coenzyme R [5] for rhizobium (*1*, *2*) and evidence for the existence of another essential growth factor. The tests consisted in the addition to a nutrient solution, containing ample quantities of all mineral constituents, of many organic nitrogen compounds and of salts of 77 chemical elements. These compounds were unable to replace the need by rhizobium for both coenzyme R and the second growth factor ("rhizobiosin") in an unpurified solution. Neither could one of these compounds, totaling more than 140, be substituted for rhizobiosin when added with nutrose to a purified solution. The possibility that an inorganic deficiency is responsible for the results attributed to rhizobiosin is quite slight. The possibility that the addition of 100 mg of magnesium carbonate per liter in the purification resulted in the formation of a soluble toxic substance is likewise remote. The addition of magnesium carbonate to the nutrient solution without subsequent filtration does not necessarily diminish growth and may cause an increase.

The presence of many mineral impurities in the crude growth-factor preparations is shown in the tabulation on page 469. The improvement in results on the trace-element requirements of rhizobium by purification of the bactopeptone preparation has also been shown. It is evident, therefore, that unless precautions are taken the increase in yield brought about by a crude preparation may be due to inorganic constituents as well as growth factors.

[5] According to verbal information from F. E. Allison (*1*, *2*), the accessory growth factor in nutrose is coenzyme R.

The data of table 3 should be interpreted with caution in view of the probable need of two accessory growth factors by rhizobium since the values may depend to a considerable extent on a selective extraction of one or other of the necessary accessories. Though no attempt was made to ascertain the existence of selective absorption by these solvents, the possibility exists.

The solutions used in the culture of azotobacter were of known composition and free from sediment or turbidity. As with aspergillus, purification with magnesium or calcium carbonate gave the best results in tests of trace-element requirements if hot filtration was employed. Hot filtration, however, tended to remove excessive quantities of phosphate and magnesium from the solution, associated perhaps with the absence of ammonium salt. Cold filtration gave better growth of the control, but poorer results on trace-element requirements. As with aspergillus, purification with calcium carbonate seemed better on the whole than that with magnesium carbonate, and the results of deficiency tests with iron were better than those with manganese or molybdenum. Again, as with aspergillus increased growth was usually paralleled by increased acidity. It should not be overlooked, in addition, that though, with increased aeration, the increased growth will probably necessitate higher concentrations of mineral constituents under conditions for maximum growth, the preparation of a simple optimum solution free from turbidity appears to be practical with potassium phosphate, magnesium sulphate, and perhaps sodium or potassium bicarbonate. The marked decreases in yield following nonaddition of molybdenum that have been reported by Bortels (*3*) and Burk and Lineweaver (*5*) have not been duplicated, however. Though the factors responsible have not been determined, it would not be illogical to assume that the trace of molybdenum in the sucrose (about 0.008 mg per 20 g sucrose), found to be present in studies with aspergillus (*15*), plays a large part.

The results obtained with nutrient-solution purification must be realized to be a first trial of the method with bacteria, and subject to improvement with further study. Extraction of the sucrose with 95-percent alcohol after the manner employed for aspergillus should also lead to improved results, particularly with molybdenum. Additional betterment in results with azotobacter should follow at higher aeration levels, with longer growth periods, and through use of strains with relatively low oxygen optima. Another important factor is the freedom from mineral impurities of the accessory growth factors with rhizobium.

SUMMARY

The use of nutrient-solution purification led to a slight improvement in results in studies of the trace-element requirements of *Rhizobium trifolii* Dangeard and *Azotobacter chroococcum* Beij., even at low nutrition levels. The results obtained were quite similar in general to those previously reported for aspergillus. No evidence could be obtained that the need of these bacteria for an essential element is not specific and almost absolute. Evidence was obtained affording additional proof of the essentiality of coenzyme R for growth of rhizobium. The necessity is also indicated of a second accessory growth factor for which the name "rhizobiosin" is proposed.

LITERATURE CITED

(1) ALLISON, FRANKLIN E., and HOOVER, SAM R.
1934. AN ACCESSORY FACTOR FOR LEGUME NODULE BACTERIA. SOURCES AND ACTIVITY. Jour. Bact. 27: 561–581.
(2) ——— HOOVER, SAM R., and BURK, DEAN.
1933. A RESPIRATION COENZYME. Science 78: 217–218.
(3) BORTELS, H.
1936. WEITERE UNTERSUCHUNGEN ÜBER DIE BEDEUTUNG VON MOLYBDÄN, VANADIUM, WOLFRAM UND ANDEREN ERDASCHENSTOFFEN FÜR STICKSTOFFBINDENDE UND ANDERE MIKROORGANISMEN. Zentbl. Bakt., [etc.] (II) 95: 193–218.
(4) BURK, DEAN.
1934. AZOTASE AND NITROGENASE IN AZOTOBACTER. Ergeb. Enzymforschung 3: 23–56.
(5) ——— and LINEWEAVER, HANS.
1931. THE INFLUENCE OF CALCIUM AND STRONTIUM UPON THE CATALYSIS OF NITROGEN FIXATION BY AZOTOBACTER. Arch. Mikrobiol. 2: [155]–186, illus.
(6) BUSTON, HAROLD WILLIAM, and PRAMANIK, BIRENDRA NATH.
1931. THE ACCESSORY FACTOR NECESSARY FOR THE GROWTH OF NEMATOSPORA GOSSYPII. THE CHEMICAL NATURE OF THE ACCESSORY FACTOR. Biochem. Jour. 25: [1656]–1670.
(7) CLARK, D. G.
1936. PHYSIOLOGICAL STUDIES ON RHIZOBIUM SPECIES. N. Y. (Cornell) Agr. Expt. Sta. Mem. 196, 30 pp.
(8) HORNER, C. KENNETH, and BURK, DEAN.
1934. MAGNESIUM, CALCIUM, AND IRON REQUIREMENTS FOR GROWTH OF AZOTOBACTER IN FREE AND FIXED NITROGEN. Jour. Agr. Research 48: 981–995, illus.
(9) KNIGHT, BERT CYRIL JAMES GABRIEL.
1937. THE NUTRITION OF STAPHYLOCOCCUS; NICTINIC ACID AND VITAMIN B_1. Biochem. Jour. 31: 731–737.
(10) KÖGL, F., and TÖNNIS, B.
1936. ÜBER DAS BIOS-PROBLEM. DARSTELLUNG VON KRYSTALLSIERTEN BIOTIN AUS EIGELB. 20. MITTEILUNG ÜBER PFLANZLICHE WACHSTUMSTOFFE. Hoppe-Seyler's Ztschr. Physiol. Chem. 242: 43–73.
(11) KOSTYTSCHEW, S., RYSKALTSCHUK, A., and SCHWEZOWA, O.
1926. BIOCHEMISCHE UNTERSUCHUNGEN ÜBER AZOTOBACTER AGILE. Hoppe-Seyler's Ztschr. Physiol. Chem. 154: 1–17.
(12) MUELLER, J. H.
1937. NICOTINIC ACID AS A GROWTH FACTOR FOR THE DIPHTHERIA BACILLUS. Jour. Biol. Chem. 120: 219–224.
(13) SCHOPFER, W. H.
1935. FACTEURS DE CROISSANCE ET VITAMINES CHEZ LES CHAMPIGNONS. Mitt. Naturf. Gesell. Bern. 134: xxxv–xxxvii.
(14) STEINBERG, ROBERT A.
1935. NUTRIENT-SOLUTION PURIFICATION FOR REMOVAL OF HEAVY METALS IN DEFICIENCY INVESTIGATIONS WITH ASPERGILLUS NIGER. Jour. Agr. Research 51: 413–424.
(15) ———
1936. RELATION OF ACCESSORY GROWTH SUBSTANCES TO HEAVY METALS, INCLUDING MOLYBDENUM, IN THE NUTRITION OF ASPERGILLUS NIGER. Jour. Agr. Research 52: 439–448.
(16) ———
1937. ROLE OF MOLYBDENUM IN THE UTILIZATION OF AMMONIUM AND NITRATE NITROGEN BY ASPERGILLUS NIGER. Jour. Agr. Research 55: 891–902.
(17) THORNE, D. W., and WALKER, R. H.
1936. PHYSIOLOGICAL STUDIES ON RHIZOBIUM: VI. ACCESSORY FACTORS. Soil Sci. 42: 231–240.
(18) WILSON, P. W.
1937. SYMBIOTIC NITROGEN-FIXATION BY THE LEGUMINOSAE. Bot. Rev. 3: 365–399.

U. S. GOVERNMENT PRINTING OFFICE: 1938

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 57 WASHINGTON, D. C., OCTOBER 1, 1938 No. 7

DIGESTIBLE NUTRIENTS AND METABOLIZABLE ENERGY IN CERTAIN SILAGES, HAYS, AND MIXED RATIONS[1]

By F. W. CHRISTENSEN, *animal nutritionist*, and T. H. HOPPER, *agricultural chemist, North Dakota Agricultural Experiment Station*[2]

INTRODUCTION

Investigation of the nutritive value of livestock feeds is a study of perennial importance. The studies herein reported were made to determine the nutritive value of sweetclover-oat straw silage, sweetclover silage, sunflower silage, corn silage, first and second cuttings, of sweetclover hay, sweetclover stems, and wild oats (*Avena fatua* L.). In certain cases the feeds mentioned were supplemented. The corn silage also served as a standard of comparision in comparative feeding trials with groups of steers. All of the tests on sweetclover were on the common white variety (*Melilotus alba* Desr.).

The first digestion trials were made with sheep in the winter and spring of 1923. During the fall and winter seasons of 1923–24 and 1924–25 additional trials were made in which steers were used as experimental animals. The trials with steers included determination of the metabolizable energy in addition to digestibility of the feeds.

EXPERIMENTAL ANIMALS

Six western grade wethers were secured on the open market for the digestion trials with sheep. They were barn-fed and handled to tame them.

In the cattle-feeding trials of 1923 and 1924, four good long-yearling steers of Shorthorn-Hereford breeding were used. They were about 20 months old at the beginning and about 24 months at the end of the tests. They had been used the previous year in a study of the protein requirements of growing cattle and were therefore accustomed to the routine of metabolism trials.

In 1924–25 good steer calves in which Hereford breeding predominated were used. They were about 9 months old when the first test started and about 12 months at the close.

For purposes of identification the letters A to F, inclusive, were used for the sheep; A–22 to D–22 for the steers used in 1923–24, and A–24 to D–24 for the steers used in 1924–25.

EQUIPMENT AND COLLECTION OF EXCRETA

For the sheep a set of six individual hog-feeding crates were remodelled by equipping them with suitable mangers and 5-pound butter jars for water receptacles. The crates were large enough to permit considerable freedom in getting up and down but did not allow space for turning about.

[1] Received for publication August 6, 1937; issued October 1938.

[2] The authors acknowledge the assistance of L. L. Nesbitt, assistant agricultural chemist, in making the chemical analyses, and of R. G. Montgomery in feeding and caring for the animals.

Bags for collecting the feces were made from ordinary duck, lined with a high grade of hospital-rubber sheeting. The bags were held in place with a light harness.

The feces were collected from the bags morning and evening and the total for the 24-hour period taken to the chemical laboratory where composite samples for the period were prepared and analyzed.

The feces and urine from the steers were collected by means of rubber ducts and rubber funnels of types long used at the Institute of Animal Nutrition of the Pennsylvania State College. The visible excreta were weighed daily and samples taken to the chemical laboratory for analysis.

WEIGHING AND SAMPLING OF FEEDS AND EXCRETA

The sheep rations for the entire day were weighed up in the morning and approximately one-half was fed in the morning and the remainder in the evening. It was soon found that individual sheep differed greatly in their likes and dislikes for the silages and hays and also in their capacities for feed consumption. Attempts to feed uniform rations to the sheep on a given feed proved futile, consequently an attempt was made to adjust the rations as nearly as possible to the appetite of the individual sheep. This attempt was only partially successful, as is evidenced by the rations offered and the feed residues collected.

In the trials with steers the dry hays and concentrates were weighed up in advance for the entire trial and samples were taken at that time. The hays were weighed into canvas bags and the concentrates into covered lard cans. When the silages were fed, enough was weighed out for each day and approximately one-half was fed in the morning and the other half in the evening. Samples of the silage were taken daily as the rations were weighed out. All samples were immediately taken to the chemical laboratory for composite sampling and analysis.

In the sheep trials the feeds and feces were weighed to the nearest gram, but in the trials with steers scales with beams graduated in tenths and hundredths of a pound were used and feeds and excreta were weighed to hundredths of a pound.

METHODS OF CHEMICAL ANALYSIS

The chemical analyses of the feeds, feed residues, and feces for moisture, ash, crude protein (total nitrogen $\times$ 6.25), true protein (albuminoid nitrogen $\times$ 6.25), ether extract, and crude fiber, and analyses of the urines for total nitrogen were made by the official methods of the Association of Official Agricultural Chemists. The total fecal excrement from the sheep trials were air-dried in a bulk-sample drier (*5*) [3] and sampled for analysis after grinding. The fresh urine and fecal samples from the steer trials were preserved with toluene and refrigeration. The fresh steer urine and fecal samples were analyzed daily for nitrogen, and the composites for moisture and nitrogen. The fresh composite fecal samples were dried in the bulk-sample drier for further analysis.

[3] Reference is made by number (italic) to Literature Cited, p. 512.

The energy values were determined with a Parr adiabatic oxygen-bomb calorimeter observing all of the necessary corrections. Before combustion the urines were dried over sulphuric acid in vacuum desiccators, and the calorific values corrected for nitrogen lost on drying.

METABOLISM TRIALS

The metabolism trials included determinations of digestibility of the rations, and in the experiments with the steers included also nitrogen balances and energy determinations on feeds and visible excreta. The trials were 10 days in length except in some of those of 1924–25 where they were 18 days long with 9-day subperiods (a and b). The preliminary and transitional periods varied in length, but in no case was the preliminary period on the test ration less than 7 days and usually it was 10 days or more. In spite of care in adjusting the test ration to the appetites of the test animals, considerable feed was left uneaten in some of the trials.

To facilitate the general account of the experiments, a schedule including dates of the preliminary and transitional feeding periods, dates of the metabolism periods, the trial numbers, the kind of animals used and their identification letters or numbers, and the kind of rations fed is given in table 1.

TABLE 1.—*Schedule of metabolism trials*

Group of experiments and trial No.	Animal designation	Feeds	Transitional and preliminary periods [1]	Metabolism periods
Experiments of 1922–23 with sheep:				
1	A, B	Sweetclover-oat straw silage, 1922	Feb. 10–27	Feb. 28–Mar. 9.
2	D, E, F	Sweetclover silage, bud stage, 1922	do	Do.
3	A, B, C	Sunflower silage, 1922	Mar. 11–27	Mar. 28–Apr. 6.
4	D, E, F	Corn silage, 1922	do	Do.
5	A	Sweetclover hay, first cutting, 1922	Apr. 7–29	Apr. 30–May 9.
6	C, D	Sweetclover hay, second cutting, 1922	do	Do.
7	E	Sweetclover stems and corn meal, 1922	do	Do.
Experiments of 1923–24 with steers:				
8	A–22, B–22	Sweetclover silage, early bloom, 1923	Dec. 25–Jan. 15	Jan. 16–25.
9	C–22, D–22	Sweetclover silage, early bloom, 1923, alfalfa, and linseed meal	do	Do.
10	A–22, B–22	Sweetclover silage, full bloom, 1923	Jan. 26–Feb. 5	Feb. 6–15.
11	C–22, D–22	Sweetclover silage, full bloom, 1923, alfalfa, and linseed meal	do	Do.
12	A–22, B–22	Sunflower silage, 1923, alfalfa, and linseed meal	Feb. 16–Mar. 11	Mar. 12–21
13	C–22	Sunflower silage, 1923	do	Do.
14	A–22, B–22	Corn silage, 1923, alfalfa, and linseed meal	Mar. 22–Apr. 1	Apr. 2–11.
15	C–22, D–22	Corn silage, 1923	do	Do.
16	A–22, B–22	Alfalfa hay, 1923	Apr. 14–29	Apr. 30–May 9.
17	C–22, D–22	Alfalfa hay, 1923, and linseed meal	do	Do.
Experiments of 1924–25 with steers:				
18	A–24, B–24, C–24, D–24	Alfalfa hay, 1924	Nov. 12–Dec. 9	Dec. 10–19.
19	A–24, B–24, C–24, D–24	Sweetclover silage, early bloom, 1924, alfalfa, barley, and linseed meal	Dec. 20–Feb. 8	Feb. 9–26.[2]
20	A–24, B–24, C–24, D–24	Sweetclover silage, early bloom, 1924	Feb. 27–Mar. 15	Mar. 16–Apr. 2.[2]
21	A–24, B–24	Wild oats, alfalfa hay	Apr. 3–May 2	May 3–22.[3]
22	C–24, D–24	Alfalfa hay	do	Do.

[1] Dates are inclusive.
[2] These 18-day periods were divided into subperiods of 9 days each.
[3] This 20-day period was divided into subperiods of 10 days each.

DESCRIPTION OF FEEDS

The same general method was used each year in preparing the silage. The crops were chopped with the usual equipment and thoroughly packed in 25-ton concrete stave silos. About every other day for a week or 10 days after filling, water was sprinkled over the silage, and the material thoroughly packed at the top by tamping. The sweetclover was cut with a binder, leaving a stubble 8 to 10 inches high.

One year the inside of the silos was coated with paraffin in an experiment to determine whether or not this treatment would make it easier to remove the frozen silage from the walls. There was no apparent advantage from this treatment. Paraffin was also used for sealing the doors and the top of the silo. Sealing the doors with paraffin proved helpful in preventing spoilage around the doors of the experimental silos and it has been used regularly in later years. For sealing the silage at the top the paraffin proved unsatisfactory. Cracks formed in the paraffin as the silage settled and about the usual amount of spoilage occurred.

SWEETCLOVER-OAT STRAW SILAGE (BUD STAGE)

The sweetclover for the sweetclover-oat straw silage fed in trial 1 was cut in the bud stage, when the plants were about 36 inches high. The freshly cut material as ensiled contained 17.6 percent of dry matter. The water content was reduced by adding, as the sweetclover was chopped, 1 part of dry oat (*Avena sativa* L.) straw to 5.5 parts of sweetclover. Considerable difficulty was experienced in obtaining a uniform mixture of the wet sweetclover and the dry oat straw, resulting in some moldy spots found occasionally throughout the silo. These moldy portions were discarded so far as possible in feeding.

SWEETCLOVER SILAGE (BUD STAGE)

The silage made from sweetclover cut in the bud stage of growth was fed in trial 2. The moisture content was reduced by wilting and the material as ensiled contained 34.8 percent of dry matter. Unfavorable weather conditions delayed ensiling 14 days after cutting. The bundles were shocked and the sweetclover was in good condition in spite of the adverse weather conditions.

SWEETCLOVER SILAGE (EARLY BLOOM STAGE)

The sweetclover silage of 1923, fed in trials 8 and 9, was made from sweetclover cut when the plants were 5 to 10 percent in blossom. The sweetclover was allowed to wilt in the field and when ensiled the material contained 33.9 percent of dry matter.

The sweetclover for the 1924 early bloom stage of growth silage, which was fed in trials 19 and 20, was allowed to wilt in the field until the leaves on the outer portion of the bundles were either dried or very much wilted and the stems considerably shrunken. Rain delayed ensiling. The first portion ensiled contained 22.1 percent and the second 26.5 percent of dry matter. The resulting silage was well-preserved, having an olive-green color and a strong sweetclover odor. In appearance and palatability it was better than the other sweetclover silages.

SWEETCLOVER SILAGE (FULL BLOOM STAGE)

The sweetclover for the silage of 1924, which was fed in trials 10 and 11, was cut in full bloom and allowed to wilt in the field. When ensiled, it contained 42.8 percent of dry matter.

SUNFLOWER SILAGE

The Mammoth Russian sunflowers (*Helianthus annuus* L.) for the silage made in 1922 and fed in trial 3 were cut when a count showed 85 percent of the plants were in blossom, 3 percent had dropped their petals, and 12 percent were in bud. The material as ensiled contained 21.4 percent of dry matter.

The Mammoth Russian sunflowers for the silage made in 1923 and fed in trials 12 and 13 were cut immediately after a killing frost when 75 percent of the plants had reached or passed the blossom stage of growth. Rain delayed ensiling until the third and sixth days after cutting. The leaves were all well-dried or rusted when the plants were cut, and moldy spots were noted on some of the coarse, woody stalks at the time of ensiling. The material when ensiled contained 31.8 percent of dry matter.

CORN SILAGE

The Mercer flint corn (*Zea mays* L.) for the silage made in 1922 and fed in trial 4 was cut in the late milk to early dough stage of growth. The plants were from 7 to 9 feet high and were ensiled without much wilting. The ears represented 40 percent and the stover 60 percent of the dry matter in the fodder. As ensiled the fodder contained 24.7 percent of dry matter.

The Mercer flint corn for the silage made in 1923 and fed in trials 14 and 15 was cut in the glazed stage of growth and as ensiled contained 38.1 percent of dry matter.

SWEETCLOVER HAY AND STEMS

The sweetclover hays from the first cutting (trial 5) and from the second cutting (trial 6) were cut in the early blossom stage of growth. The hays were ground in a hammer mill, through a hay screen, reducing the leaves largely to a powder and the stems to particles one-half inch or less in length. The hays were both good, but a little coarse-stemmed.

The sweetclover stems were those left after sheep had eaten practically all of the leaves and fine stems from hay cut in the early blossom stage of growth. The stems were ground in the hammer mill and fed to two sheep in a digestion trial (7), but only one sheep ate enough to complete the trial period, even when supplemented with corn.

ALFALFA HAY

The alfalfa hay for the 1923–24 trials (9, 11, 12, 14, 16, and 17) was of fairly good quality but was rather coarse-stemmed with a fair amount of leaves but somewhat lacking in color.

The alfalfa (*Medicago sativa* L.) for the 1924–25 trials (18, 19, 21, and 22) was somewhat better, being leafier, finer-stemmed and having a better color.

CONCENTRATES

The linseed meal was the standard old-process product.

The barley (*Hordeum vulgare* L.) (trial 19) and corn meal (trial 7) were of good feed grade.

The wild oats [4] (fed in trial 21), which are removed in cleaning wheat for milling and commonly ground and sold as "mill oats," were found on analysis [5] to consist of 84.4 percent of wild oats, mostly dark color, 9.2 percent of cultivated oats, 4.9 percent of wheat (*Triticum aestivum* L.), 1.5 percent of barley, and a trace of rye (*Secale cereale* L.). They had a test weight of 37.5 pounds per bushel and a separation showed 65 percent of kernel and 35 percent of hulls by weight.

COMPOSITION OF CROPS AND FEEDS

The chemical composition of each of the green silage crops and the resulting silages, and of the alfalfa hay, sweetclover hay, sweetclover stems, and concentrates is given in table 2. The trial in which the feeds were fed is indicated by the trial number.

[4] The wild oats were from the same lot as those fed by Griswold ((*4*), *p. 12*).
[5] Analysis by P. J. Olson, assistant agronomist, North Dakota Agricultural Experiment Station.

TABLE 2.—*Chemical composition of the green silage crops and the resulting silages, and of the alfalfa and sweetclover hays, the sweetclover stems, and the concentrates fed*

Hay, feed, or silage	Year or trial No.	Dry matter	Composition of dry matter							
			Ash	Crude protein	Crude fiber	N-free extract	Ether extract	Energy per 100 pounds	Protein nitrogen	Non-protein nitrogen
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Therms*	*Percent*	*Percent*
Crops when ensiled:										
Sweetclover-oat straw:										
Sweetclover, bud stage	1922	17.59	9.91	19.90	25.75	42.55	1.91			
Oat straw	1922	83.90	8.88	6.34	40.58	42.03	2.17			
Sweetclover, bud stage	1922	34.75	9.16	21.21	29.60	38.02	2.01			
Sweetclover, early bloom	1923	33.91	8.91	17.64	32.76	38.73	1.96			
Sweetclover, full bloom	1923	42.77	8.86	19.62	33.54	35.84	2.14		1.886	0.937
Sweetclover, early bloom, silo 1	1924	22.12	10.41	22.31	29.08	35.40	2.80		1.920	1.219
Sweetclover, early bloom, silo 2	1924	26.45	9.65	21.13	29.42	36.72	3.08		2.132	1.437
Average		24.29	10.03	21.72	29.25	36.06	2.94		1,836	1.545
Sunflowers	1922	21.40	9.88	9.34	30.30	49.02	1.46		1.984	1.491
Corn fodder, green	1923	31.80	12.68	9.62	33.33	42.14	2.23			
	1922	24.71	7.62	8.36	20.82	61.01	2.19		1.150	.389
	1923	38.07	5.09	8.10	16.82	67.00	2.99			
Silages:									1.117	.179
Sweetclover-oat straw	1	21.97	10.96	14.16	41.22	29.05	4.61			
Sweetclover, bud stage (1922)	2	34.78	9.70	21.53	35.71	29.76	3.30			
Sweetclover, early bloom (1923)	8 and 9	33.47	9.21	18.33	29.91	39.47	3.09	212.14	1.012	1.921
Sweetclover, full bloom (1923)	10 and 11	32.66	9.95	21.36	32.00	33.97	2.72	210.16	1.350	2.067
Sweetclover, early bloom, subperiod a, 1924	19	23.84	10.24	20.72	33.57	31.96	3.51	209.68	1.077	2.238
Sweetclover, early bloom, subperiod b, 1924	19	24.21	10.22	21.24	32.40	32.64	3.50	207.61	1.043	2.356
Average		24.02	10.23	20.98	32.98	32.30	3.50	208.64	1.060	2.297
Sweetclover, early bloom, subperiod a, 1924	20	24.85	9.98	22.47	31.02	32.74	3.79	211.55	1.541	2.054
Sweetclover, early bloom, subperiod b, 1924	20	24.55	10.09	22.44	31.28	32.34	3.85	212.30	1.565	2.026
Average		24.70	10.04	22.46	31.15	32.54	3.82	211.92	1.553	2.040
Average of all sweetclover		28.34	9.91	21.16	32.27	33.27	3.39	210.57	1.265	2.110
Sunflowers (1922)	3	26.18	9.95	10.20	32.58	44.49	2.78			
Sunflowers (1923)	12 and 13	26.05	15.00	8.20	38.15	35.55	2.10	188.29	.797	.515
Average		26.12	12.48	9.20	35.37	40.52	2.44			

Corn (1922)	4	24.35	7.86	10.16	22.89	56.36	2.73			
Corn (1923)	14 and 15	30.47	7.09	8.29	22.48	59.36	2.78	201.66	.786	.540
Average		27.41	7.48	9.23	22.69	57.86	2.76			
Alfalfa hay	9	84.72	10.62	15.98	34.64	37.15	1.61	199.72	1.896	.660
	11	83.78	10.55	16.54	35.41	35.85	1.65	199.66	1.917	.730
	12	86.02	10.25	16.19	35.66	36.45	1.45	197.88	1.885	.706
	14	86.40	9.44	14.98	39.03	35.17	1.38	199.53	1.724	.673
	16 and 17	85.59	10.40	16.68	35.62	36.05	1.25	200.50	1.922	.747
	18	84.81	9.90	17.16	32.18	38.48	2.28	199.74	2.183	.563
	19	84.49	9.71	17.38	32.05	38.57	2.29	202.65	2.166	.615
	21 and 22	85.34	9.49	16.79	33.91	37.98	1.83	201.92	2.119	.568
Sweetclover hay, first cutting, ground	5	82.46	10.50	16.27	38.82	32.65	1.76			
Sweetclover hay, second cutting, ground	6	82.58	7.36	18.46	36.15	36.30	1.73			
Sweetclover stems, ground	7	85.33	6.28	9.42	52.29	31.04	.97			
Composition of concentrates fed with hays and silages:										
Corn meal	7	85.54	2.04	10.90	3.50	79.67	3.89			
Linseed meal	9	88.76	6.73	39.01	9.37	37.50	7.39	222.73	5.870	.371
	11	88.55	6.74	39.48	9.31	37.01	7.46	221.72	5.759	.557
	12	88.04	6.76	39.86	9.43	36.74	7.21	223.24	5.946	.432
	14	88.51	6.82	39.64	9.06	37.63	6.85	223.88	5.729	.613
	17	88.57	6.65	39.66	9.63	37.17	6.89	224.39	5.656	.690
	19	85.87	5.42	37.49	9.61	40.94	6.54		5.713	.285
Barley, ground	19	85.96	3.41	12.47	5.81	76.06	2.25		1.915	.080
Grain mixture, (ground barley 3 parts, linseed meal 1 part)	19	86.65	3.98	19.33	7.42	66.17	3.10	206.81	2.892	.201
Wild oats	21	88.07	4.41	13.30	15.50	60.77	6.02	214.03	1.893	.235

It is not possible to make close comparisons between the composition of the crop as it was ensiled and the resulting silage because the samples of the crops were taken to represent the material as it was put into the silo, either as a whole or as definite portions of the whole. The samples taken for the digestion trials represent only the silage fed during the trials, and, consequently, they represent a rather limited portion of the whole. An inspection of the data, however, shows some outstanding differences.

The dry-matter content of the crops as compared to the silages varied considerably in some years and very little in others. These variations probably resulted chiefly from differences in the quantities of water added at the top of the silos in packing the material and from leakages at the bottom. A comparison of the composition of the dry matter indicates that, in general, the ash was lower in the crop than in the silage. Similarly, the protein was usually lower in the crop than in the silage, excepting the sunflowers of 1923 where the protein was 1.42 percent higher in the crop than in the silage. With the exception mentioned, the protein ranged from 0.19 to 1.80 percent lower in the crop than in the silage. The crude fiber in the sweetclover of 1923 was 1.54 to 2.85 percent higher in the crop than in the silage, but in all of the other silages the crude fiber ranged from 0.21 to 6.11 percent lower in the crop than in the silage. The nitrogen-free extract ranged from 1.87 to 8.26 percent higher in the crop than in the silage, except in the early cutting of sweetclover, 1923, which showed 0.74 percent less in the crop. The ether extract in the silage ranged from 0.54 to 1.32 percent higher than the crop, except in the sunflower silage, 1923, and the corn silage, 1923, where it was 0.13 and 0.21 percent lower, respectively.

A comparison of the average composition of seven samples of sweetclover silage with the average of two samples of corn silage shows that the sweetclover silages contained 2.43 percent more ash, 11.93 percent more crude protein, 9.58 percent more crude fiber and 0.63 percent more ether extract, but 24.59 percent less of nitrogen-free extract.

A similar comparison of the average of two sunflower silages with the two corn silages shows that the sunflower silage contained nearly the same amounts of protein and nonprotein nitrogen and ether extract as the corn silage, but it contained 5.00 percent more ash and 12.68 percent more crude fiber and 17.34 percent less of nitrogen-free extract. The sunflower silages averaged 3.1 percent higher in crude fiber than the sweetclover silages.

PROTEIN NITROGEN IN PERCENT OF TOTAL NITROGEN IN CROPS AND IN SILAGES

Determinations of protein and nonprotein nitrogen made on a number of the crops and silages show that considerable amounts of protein were broken down into the nonprotein form. The extent of these changes is shown in table 3. In this table the protein nitrogen is given in percentage of the total. The protein nitrogen in the crops ranged from 57.02 to 86.19 percent of the total nitrogen. In the silages the protein nitrogen ranged from 34.50 to 60.75 percent. The changes from protein to nonprotein nitrogen ranged from 13.97 percent in the sunflowers to 32.31 percent in the early bloom sweetclover of 1923.

TABLE 3.—*Protein nitrogen as percentage of total nitrogen in crops and in silages*

Kind of crop	Crop		Silage		Difference
	Year	Percent	Trial No.	Percent	
					Percent
Sweetclover, early bloom	1923	66.81	1–23	34.50	32.31
Sweetclover, full bloom	1923	61.17	2–23	39.51	21.66
Sweetclover, early bloom	1924	[1] 57.02	3–24	[2] 37.41	19.61
Sunflower	1923	[1] 74.72	3–23	60.75	13.97
Corn fodder, green	1923	86.19	4–23	59.28	26.91

[1] Average of 2 analyses.
[2] Average of 4 analyses.

DIGESTIBILITY OF THE SILAGES

The rations fed, feed residues and feces collected, and the detailed data on the digestibility of the nutrients in the silages are given in table 4. Data showing the composition of the feed residues and feces in the trials are omitted. Their composition, if desired, can readily be computed from the tables on digestibility. The data on the digestibility of other feeds and rations are given in succeeding tables. The digestion coefficients determined in the trials are summarized in table 7 and a discussion of the results appears in connection with it.

TABLE 4.—*Digestibility of sweetclover-oat straw silage, sweetclover silage, sunflower silage, corn silage, alfalfa and sweetclover hays, and sweetclover stems, when fed to sheep and steers*

Trial No.	Animal	Feeds and other items	Original weight	Dry matter	Organic matter	Ash	Crude protein	Crude fiber	N-free extract	Ether extract
		Sweetclover-oat straw silage:								
		Silage, bud stage—grams	1,585.0	348.2	310.0	38.2	49.3	143.5	101.2	16.1
		Feed residue—do	81.2	74.9	67.5	7.4	9.5	28.3	28.0	1.7
1	Sheep A	Total eaten—do		273.3	242.5	30.8	39.8	115.2	73.2	14.4
		Feces—do		188.4	165.2	23.2	19.4	71.4	68.6	5.8
		Digested—do	445.5	84.9	77.3	7.6	20.4	43.8	4.6	8.6
		——do—percent		31.06	31.88	24.68	51.26	38.02	6.28	59.72
		Silage, bud stage—grams	1,133.0	248.9	221.6	27.3	35.2	102.6	72.3	11.5
		Feed residue—do	12.7	12.0	10.8	1.2	1.2	4.9	4.4	.2
1	Sheep B	Total eaten—do		236.9	210.8	26.1	34.0	97.7	67.9	11.3
		Feces—do	324.2	148.7	129.2	19.5	16.4	52.8	54.5	5.6
		Digested—do		88.2	81.6	6.6	17.6	44.9	13.4	5.7
		——do—percent		37.23	38.71	25.29	51.76	45.96	19.73	50.44
		Sweetclover silage:								
		Silage, bud stage—grams	680.0	236.5	213.6	22.9	50.9	84.5	70.4	7.8
		Feed residue—do	128.5	120.3	109.8	10.5	24.0	48.4	35.4	1.9
2	Sheep D	Total eaten—do		116.2	103.8	12.4	26.9	36.1	35.0	5.9
		Feces—do	160.1	58.1	52.5	5.6	7.3	27.6	15.5	2.1
		Digested—do		58.1	51.3	6.8	19.6	8.5	19.5	3.8
		——do—percent		50.00	49.42	54.84	72.86	23.55	55.71	64.41
		Silage, bud stage—grams	1,586.0	551.6	498.1	53.5	118.8	197.0	164.2	18.2
		Feed residue—do	12.7	11.9	10.9	1.0	2.0	5.4	3.4	.1
2	Sheep E	Total eaten—do		539.7	487.2	52.5	116.8	191.6	160.8	18.1
		Feces—do	697.7	225.4	205.2	20.2	26.3	106.1	66.0	6.8
		Digested—do		314.3	282.0	32.3	90.5	85.5	94.8	11.3
		——do—percent		58.24	57.88	61.52	77.48	44.62	58.96	62.43
		Silage, bud stage—grams	680.0	236.5	213.6	22.9	50.9	84.5	70.4	7.8
		Feed residue—do	67.4	63.5	58.0	5.5	10.9	26.9	19.6	.6
2	Sheep F	Total eaten—do		173.0	155.6	17.4	40.0	57.6	50.8	7.2
		Feces—do	198.9	73.8	66.2	7.6	9.1	32.4	22.1	2.7
		Digested—do		99.2	89.4	9.8	30.9	25.2	28.7	4.5
		——do—percent		57.34	57.46	56.32	77.25	43.75	56.50	62.50
		Silage, early bloom—pounds	40.000	13.388	12.156	1.232	2.454	4.004	5.284	.414
		Feed residue—do	1.018	.016	.014	.002	.003	.006	.005	.000
8	Steer A–22	Total eaten—do		13.372	12.142	1.230	2.451	3.998	5.279	.414
		Feces—do	39.982	5.478	4.996	.482	.721	2.485	1.614	.176
		Digested—do	26.999	7.894	7.146	.748	1.730	1.513	3.665	.238
		——do—percent		59.02	58.85	60.81	70.58	37.84	69.43	57.49
		Silage, early bloom—pounds	40.000	13.388	12.156	1.232	2.454	4.004	5.284	.414
		Feed residue—do	1.009	.018	.016	.002	.003	.007	.006	.001
8	Steer B–22	Total eaten—do		13.370	12.140	1.230	2.451	3.997	5.278	.413
		Feces—do	39.991	5.565	5.047	.518	.767	2.456	1.658	.166
		Digested—do	28.654	7.805	7.093	.712	1.684	1.541	3.820	.247
		——do—percent		58.38	58.43	57.89	68.71	38.55	68.59	59.81

		Silage, full bloom—pounds	40.000	13.064	11.764	1.300	2.790	4.180	4.438	.355
		Feed residue—do	[1].646	.580	.522	.058	.130	.206	.171	.015
10	Steer A-22	Total eaten—do	39.354	12.484	11.242	1.242	2.660	3.974	4.267	.340
		Feces—do	23.805	4.759	4.314	.445	.672	2.166	1.316	.160
		Digested—do		7.725	6.928	.797	1.998	1.808	2.951	.180
		do—percent		61.88	61.63	64.17	74.74	45.50	69.16	52.94
		Silage, full bloom—pounds	40.000	13.064	11.764	1.300	2.791	4.181	4.438	.355
10	Steer B-22	Feces—do	29.988	5.455	4.963	.492	.785	2.446	1.534	.197
		Digested—do		7.609	6.801	.808	2.005	1.734	2.904	.158
		do—percent		58.24	57.81	62.15	71.86	41.48	65.43	44.51
		Silage, early bloom—pounds	26.000	6.461	5.816	.645	1.452	2.004	2.115	.245
20, subperiod *a*	Steer A-24	Feces—do	13.909	2.356	2.107	.249	.292	1.173	.556	.086
		Digested—do		4.105	3.709	.396	1.160	.831	1.559	.159
		do—percent		63.54	63.77	61.40	79.89	41.47	73.71	64.90
		Silage, early bloom—pounds	26.000	6.383	5.739	.644	1.432	1.997	2.064	.246
20, subperiod *b*	Steer A-24	Feces—do	13.910	2.316	2.051	.265	.294	1.122	.551	.084
		Digested—do		4.067	3.688	.379	1.138	.875	1.513	.162
		do—percent		63.72	64.26	58.85	79.47	43.82	73.30	65.85
		Silage, early bloom—pounds	26.000	6.461	5.816	.645	1.452	2.004	2.115	.245
20, subperiod *a*	Steer B-24	Feces—do	12.991	2.316	2.089	.227	.289	1.166	5.42	.092
		Digested—do		4.145	3.727	.418	1.163	.838	1.573	.153
		do—percent		64.15	64.08	64.81	80.10	41.82	74.37	62.45
		Silage, early bloom—pounds	26.000	6.383	5.739	.644	1.432	1.997	2.064	.246
20, subperiod *b*	Steer B-24	Feces—do	12.582	2.294	2.033	.260	.301	1.113	.549	.071
		Digested—do		4.089	3.706	.384	1.131	.884	1.515	.175
		do—percent		64.06	64.58	59.63	78.98	44.26	73.40	71.14
		Silage, early bloom—pounds	28.000	6.958	6.264	.694	1.564	2.158	2.278	.264
20, subperiod *a*	Steer C-24	Feces—do	17.296	2.518	2.267	.251	.321	1.249	.609	.088
		Digested—do		4.440	3.997	.443	1.243	.909	1.669	.176
		do—percent		63.81	63.81	63.83	79.48	42.12	73.27	66.67
		Silage, early bloom—pounds	28.000	6.874	6.180	.694	1.543	2.150	2.223	.265
20, subperiod *b*	Steer C-24	Feces—do	16.240	2.509	2.237	.272	.318	1.201	.628	.090
		Digested—do		4.365	3.943	4.22	1.225	.949	1.595	.175
		do—percent		63.50	63.80	60.81	79.39	44.14	71.75	66.04
		Silage, early bloom—pounds	28.000	6.958	6.264	.694	1.564	2.158	2.278	.264
20, subperiod *a*	Steer D-24	Feces—do	15.507	2.571	2.312	2.60	3.22	1.272	.629	.088
		Digested—do		4.387	3.952	.434	1.242	.886	1.649	.176
		do—percent		63.05	63.09	62.54	79.42	41.06	72.39	66.67
		Silage, early bloom—pounds	28.000	6.874	6.180	.694	1.543	2.150	2.223	.265
20, subperiod *b*	Steer D-24	Feces—do	14.551	2.468	21.83	.285	.312	1.204	.578	.089
		Digested—do		4.406	3.997	.409	1.231	.946	1.645	.176
		do—percent		64.10	64.68	58.93	79.78	44.00	74.00	66.42
		Sunflower silage:								
		Silage—grams	2,500.0	654.5	589.4	65.1	66.8	213.2	291.2	18.2
		Feed residue—do	[1] 213.0	198.9	182.8	16.1	12.5	95.7	72.1	2.6
3	Sheep A	Total eaten—do		455.6	406.6	49.0	54.3	117.5	219.1	15.6
		Feces—do	[1] 232.0	207.3	182.0	25.3	25.2	70.6	81.2	4.9
		Digested—do		248.3	224.6	23.7	29.1	46.9	137.9	10.7
		do—percent		54.50	55.24	48.37	53.59	39.91	62.94	68.59

TABLE 4.—*Digestibility of sweetclover-oat straw silage, sweetclover silage, sunflower silage, corn silage, alfalfa and sweetclover hays, and sweetclover stems, when fed to sheep and steers*—Continued

Trial No.	Animal	Feeds and other items	Original weight	Dry matter	Organic matter	Ash	Crude protein	Crude fiber	N-free extract	Ether extract
		Sunflower silage—Continued.								
		Silage, grams	1,815.0	475.2	427.9	47.3	48.5	154.8	211.4	13.2
		Feed residue, do	[1] 117.1	108.9	98.6	10.3	9.5	41.9	44.7	2.5
		Total eaten, do		366.3	329.3	37.0	39.0	112.9	166.7	10.7
3	Sheep B	Feces, do	[1] 205.0	183.7	163.1	20.6	21.1	66.8	71.3	3.9
		Digested, do		182.6	166.2	16.4	17.9	46.1	95.4	6.8
		do, percent		49.85	50.47	44.32	45.90	40.83	57.23	63.55
		Silage, grams	1,815.0	475.2	427.9	47.3	48.5	154.8	211.4	13.2
		Feed residue, do	[1] 69.4	64.5	58.2	6.3	5.8	26.9	24.2	1.4
		Total eaten, do		410.7	369.7	41.0	42.7	127.9	187.2	11.8
3	Sheep C	Feces, do	[1] 217.3	194.6	172.9	21.7	20.2	70.6	77.9	4.2
		Digested, do		216.1	196.8	19.3	22.5	57.3	109.3	7.6
		do, percent		52.62	53.23	47.07	52.69	44.80	58.39	64.41
		Silage, pounds	24.000	6.252	5.314	.938	.513	2.385	2.285	.131
		Feces, do	13.766	3.125	2.738	.387	2.88	1.438	.977	.036
13	Steer C–22	Digested, do		3.127	2.576	.551	2.25	.947	1.308	.095
		do, percent		50.02	48.48	58.74	43.86	39.71	57.24	72.52
		Corn silage:								
		Silage, grams	1,815.0	442.0	407.3	34.7	44.9	101.2	249.1	12.1
		Feed residue, do	[1] 95.2	89.7	80.2	9.5	7.8	27.8	43.1	1.6
		Total eaten, do		352.3	327.1	25.2	37.1	73.4	206.0	10.5
4	Sheep D	Feces, do	[1] 84.5	76.3	59.9	16.4	7.6	15.2	35.3	1.9
		Digested, do		276.0	267.2	8.8	29.5	58.2	170.7	8.6
		do, percent		78.34	81.69	34.92	79.51	79.29	82.86	81.90
		Silage, grams	2,500.0	608.8	560.9	47.9	61.9	139.4	343.1	16.6
		Feces, do	[1] 203.4	186.5	157.1	29.4	22.4	50.2	81.4	3.1
4	Sheep E	Digested, do		422.3	403.8	18.5	39.5	89.2	261.7	13.5
		do, percent		69.37	71.99	38.62	63.81	63.99	76.28	81.33
		Silage, grams	1,815.0	442.0	407.3	34.7	44.9	101.2	249.1	12.1
		Feed residue, do	[1] 111.7	105.6	96.9	8.7	8.8	31.1	55.5	1.5
		Total eaten, do		336.4	310.4	26.0	36.1	70.1	193.6	10.6
4	Sheep F	Feces, do	[1] 92.4	83.8	66.6	17.2	11.7	18.3	34.7	1.8
		Digested, do		252.6	243.8	8.8	24.4	51.8	158.9	8.8
		do, percent		75.09	78.54	33.85	67.59	73.89	82.08	83.02
		Silage, pounds	30.000	9.141	8.493	.648	.758	2.055	5.426	.254
		Feed residue, do	.103	.095	.088	.007	.008	.024	.053	.003
		Total eaten, do		9.046	8.405	.641	.750	2.031	5.373	.251
15	Steer C–22	Feces, do	14.311	2.899	2.519	.380	.339	.790	1.346	.044
		Digested, do	15.586	6.147	5.886	.261	.411	1.241	4.028	.207
		do, percent		67.95	70.03	40.72	54.80	61.10	74.97	82.47

15	Steer D-22	Silage—pounds	30.000	9.141	8.493	.648	.758	2.055	5.426	.254
		Feces—do	21.915	3.401	2.989	.412	.414	.939	1.583	.053
		Digested—do		5.740	5.504	2.36	.344	1.116	3.843	.201
		do—percent		62.79	64.81	36.42	45.38	54.31	70.83	79.13
		Alfalfa and sweet clover hays:								
16	Steer A-22	Alfalfa hay—pounds	11.000	9.415	8.436	.979	1.570	3.354	3.394	.118
		Feces—do	18.867	4.024	3.467	.557	.478	1.831	1.054	.104
		Digested—do		5.391	4.969	.422	1.092	1.523	2.340	.014
		do—percent		57.26	58.90	43.11	69.55	45.41	68.95	11.86
16	Steer B-22	Alfalfa hay—pounds	11.000	9.415	8.436	.979	1.570	3.354	3.394	.118
		Feces—do	23.255	4.072	3.486	.586	.483	1.826	1.062	.115
		Digested—do		5.343	4.950	.393	1.087	1.528	2.332	.003
		do—percent		56.75	58.68	40.14	69.24	45.56	68.71	2.54
18	Steer A-24	Alfalfa hay—pounds	10.000	8.481	7.641	.840	1.455	2.729	3.263	.193
		Feces—do	16.784	3.657	3.223	.434	.422	1.768	.908	.125
		Digested—do		4.824	4.418	.406	1.033	.961	2.355	.068
		do—percent		56.88	57.81	48.33	71.00	35.21	72.17	35.23
18	Steer B-24	Alfalfa hay—pounds	10.000	8.481	7.641	.840	1.455	2.729	3.263	.193
		Feces—do	16.204	3.615	3.184	.431	.440	1.742	.882	.119
		Digested—do		4.866	4.457	.409	1.015	.987	2.381	.074
		do—percent		57.38	58.33	48.69	69.76	36.17	72.97	38.34
18	Steer C-24	Alfalfa hay—pounds	10.000	8.481	7.641	.840	1.455	2.729	3.263	.193
		Feces—do	17.256	3.556	3.122	.434	.434	1.694	.865	.129
		Digested—do		4.925	4.519	.406	1.021	1.035	2.398	.064
		do—percent		58.07	59.14	48.33	70.17	37.93	73.49	33.16
18	Steer D-24	Alfalfa hay—pounds	9.000	7.633	6.877	.756	1.310	2.456	2.937	.174
		Feces—do	15.892	3.283	2.901	.382	.394	1.593	.799	.115
		Digested—do		4.350	3.976	.374	.916	.863	2.138	.059
		do—percent		56.99	57.82	49.47	69.92	35.14	72.80	33.91
22, subperiod *a*	Steer C-24	Alfalfa hay—pounds	13.000	11.094	10.041	1.053	1.863	3.762	4.213	.203
		Feces—do	21.878	4.411	3.934	.477	.543	2.149	1.096	.146
		Digested—do		6.683	6.107	.576	1.320	1.613	3.117	.057
		do—percent		60.24	60.82	54.70	70.85	42.88	73.99	28.08
22, subperiod *b*	Steer C-24	Alfalfa hay—pounds	13.000	11.094	10.041	1.053	1.863	3.762	4.213	.203
		Feces—do	23.018	4.640	4.144	.496	.559	2.349	1.091	.145
		Digested—do		6.454	5.897	.557	1.304	1.413	3.122	.058
		do—percent		58.18	58.73	52.90	69.99	37.56	74.10	28.57
22, subperiod *a*	Steer D-24	Alfalfa hay—pounds	13.000	11.094	10.041	1.053	1.863	3.762	4.213	.203
		Feces—do	23.640	4.655	4.158	.497	.552	2.304	1.150	.152
		Digested—do		6.439	5.883	.556	1.311	1.458	3.063	.051
		do—percent		58.04	58.59	52.80	70.37	38.76	72.70	25.12
22, subperiod *b*	Steer D-24	Alfalfa hay—pounds	13.000	11.094	10.041	1.053	1.863	3.762	4.213	.203
		Feces—do	23.621	4.693	4.174	.519	.560	2.323	1.132	.159
		Digested—do		6.401	5.867	.534	1.303	1.439	3.081	.044
		do—percent		57.70	58.43	50.71	69.94	38.25	73.13	21.67
5	Sheep A	Sweetclover hay, first cutting—grams	680.0	560.7	501.8	58.9	91.2	217.7	183.1	9.9
		Feces—do	266.3	242.0	225.8	16.2	22.5	127.3	69.8	6.2
		Digested—do		318.7	276.0	42.7	68.7	90.4	113.3	3.7
		do—percent		56.84	55.00	72.50	75.33	41.53	61.88	37.37

See footnotes at end of table.

TABLE 4.—*Digestibility of sweetclover-oat straw silage, sweetclover silage, sunflower silage, corn silage, alfalfa and sweetclover hays, and sweetclover stems, when fed to sheep and steers*—Continued

Trial No.	Animal	Feeds and other items	Original weight	Dry matter	Organic matter	Ash	Crude protein	Crude fiber	N-free extract	Ether extract
		Alfalfa and sweet clover hays—Continued.								
6	Sheep C	Sweetclover hay, second cutting.....grams	453.0	374.1	346.6	27.5	69.1	135.2	135.8	6.5
		Feces.....do	175.2	150.0	148.3	11.7	16.4	81.8	45.2	4.8
		Digested.....do		214.1	198.3	15.8	52.7	53.4	90.6	1.7
		....do.....percent		57.23	57.21	57.45	76.27	39.50	66.72	26.15
6	Sheep D	Sweetclover hay, second cutting.....grams	453.0	374.1	346.6	27.5	69.1	135.2	135.8	6.5
		Feces.....do	174.9	158.0	146.5	11.5	16.2	81.3	44.5	4.5
		Digested.....do		216.1	200.1	16.0	52.9	53.9	91.3	2.0
		....do.....percent		57.77	57.73	58.18	76.56	39.87	67.23	30.77
		Sweetclover stems.....grams	453.0	386.5	362.2	24.3	36.4	202.1	120.0	3.7
		Corn meal.....do	225.0	193.1	189.1	3.9	21.0	6.8	153.8	7.5
		Total eaten.....do	678.0	579.6	551.4	28.2	57.4	208.9	273.8	11.2
7	Sheep E	Feces.....do	248.7	229.2	216.9	12.3	17.1	125.5	71.2	3.1
		Digested, total ration.....do		350.4	334.5	15.9	40.3	83.4	202.6	8.1
		Corn meal digested [2].....do		173.8	171.0	2.8	15.5	3.9	144.6	7.0
		Stems digested [3].....do		176.6	163.5	13.1	24.8	79.5	57.9	1.1
		....do.....percent		45.69	45.14	53.91	68.13	39.34	48.25	29.73
		Digested, entire ration.....do		60.46	60.66	56.38	70.21	39.92	74.00	72.32

[1] Air-dried weight.
[2] Computed by use of coefficients of digestibility from Morrison (*8*).
[3] Indirectly computed.

DIGESTIBILITY OF ALFALFA HAY, SWEETCLOVER HAY, AND SWEETCLOVER STEMS

Alfalfa hay was fed alone in some trials to furnish data for comparing trials the alfalfa formed a part of the mixed rations. The trials with sheep included some tests on sweetclover hay for comparison with alfalfa and with sweetclover silage. The detailed data on these trials are given in table 4 but the discussion of the data appears later in connection with the summary table 7.

DIGESTIBILITY OF MIXED RATIONS

In the metabolism trials of 1923–24 the silages were fed alone in its digestibility with sweetclover hay and sweetclover silage. In otner certain trials and in combination with alfalfa hay and linseed meal in other trials. A ratio of 2 pounds of linseed meal to 5 pounds of alfalfa was maintained in all the trials where silages were fed. It was intended that the same ratio would be maintained in a trial with linseed meal and alfalfa alone, but this combination proved too laxative to feed in amounts sufficient for maintenance. The ratio was therefore changed to 2 pounds of linseed meal and 7 pounds of alfalfa. Two pounds of linseed meal per head furnished a surplus of protein in the rations containing the sweetclover silages, but it was fed to insure adequate protein in the rations containing corn silage.

In the trials of 1924–25, sweetclover silage was fed alone and with 2 pounds of alfalfa hay and 4 pounds of a concentrate mixture, consisting of 3 parts of ground barley and 1 part of linseed meal. The latter ration was similar to a ration fed in some other experiments.

The detailed data on the digestibility of the mixed rations are given in table 5.

100884—38——2

TABLE 5.—*Digestibility of mixed rations: Sweetclover, sunflower, and corn silages, alfalfa hay, and concentrates*

Trial No.	Animal	Feeds and other items	Original weight	Dry matter	Organic matter	Ash	Crude protein	Crude fiber	N-free extract	Ether extract
		Sweetclover silage, early bloom ---pounds	25.000	8.368	7.598	0.770	1.534	2.503	3.303	0.259
		Alfalfa hay ---do	5.000	4.236	3.786	.450	.677	1.467	1.574	.068
		Linseed meal ---do	2.000	1.775	1.656	.120	.693	.166	.666	.131
		Total ---do	32.000	14.379	13.040	1.340	2.904	4.136	5.543	.458
9	Steer C-22	Feed residue ---do	[1] 1.263	.244	.219	.026	.039	.087	.088	.004
		Total eaten ---do	31.737	14.135	12.821	1.314	2.865	4.049	5.455	.454
		Feces ---do	29.770	5.725	5.139	.586	.800	2.504	1.652	.183
		Digested ---do		8.410	7.682	.728	2.065	1.545	3.802	.270
		---do ---percent		59.50	59.92	55.40	72.08	38.16	69.72	59.69
		Sweetclover silage, early bloom ---pounds	22.000	7.363	6.686	.677	1.350	2.202	2.906	.228
		Alfalfa hay ---do	5.000	4.236	3.786	.450	.677	1.467	1.574	.068
		Linseed meal ---do	2.000	1.775	1.656	.120	.693	.166	.666	.131
		Total ---do	29.000	13.374	12.127	1.247	2.720	3.835	5.146	.427
9	Steer D-22	Feed residue ---do	[1] 1.075	.069	.061	.008	.012	.024	.023	.001
		Total eaten ---do	28.925	13.305	12.066	1.239	2.708	3.811	5.123	.426
		Feces ---do	29.038	5.328	4.762	.566	.724	2.310	1.569	.159
		Digested ---do		7.977	7.305	.673	1.984	1.501	3.554	.267
		---do ---percent		59.95	60.53	54.32	73.26	39.39	69.37	62.68
		Sweetclover silage, full bloom ---pounds	20.000	6.532	5.882	.650	1.395	2.090	2.219	.178
		Alfalfa hay ---do	5.000	4.189	3.747	.442	.693	1.483	1.502	.069
		Linseed meal ---do	2.000	1.771	1.652	.119	.699	.165	.655	.131
		Total ---do	27.000	12.492	11.281	1.211	2.787	3.738	4.376	.378
11	Steer C-22	Feed residue ---do	[2] 2.085	1.852	1.653	.199	.432	.612	.559	.050
		Total eaten ---do		10.640	9.628	1.012	2.355	3.126	3.817	.329
		Feces ---do	24.089	4.143	3.674	.469	.585	1.820	1.147	.122
		Digested ---do		6.497	5.954	.543	1.770	1.306	2.670	.207
		---do ---percent		61.06	61.84	53.66	75.16	41.78	69.95	62.92
		Sweetclover silage, full bloom ---pounds	17.000	5.552	5.000	.552	1.186	1.777	1.886	.151
		Alfalfa hay ---do	5.000	4.189	3.747	.442	.693	1.483	1.502	.069
		Linseed meal ---do	2.000	1.771	1.652	.119	.699	.165	.655	.132
		Total eaten ---do	24.000	11.512	10.399	1.113	2.578	3.425	4.043	.352
11	Steer D-22	Feces ---do	27.046	4.511	4.040	.471	.638	2.008	1.266	.128
		Digested ---do		7.001	6.359	.642	1.940	1.417	2.777	.224
		---do ---percent		60.81	61.15	57.68	75.25	41.37	68.69	63.64

19, subperiod a	Steer A-24	Sweetclover silage, early bloom, pounds	16.000	3.814	3.423	.391	.790	1.280	1.219	.134
		Alfalfa hay, do	2.000	1.690	1.526	.164	.294	.542	.652	.039
		Grain mixture [2], do	4.000	3.466	3.328	.138	.670	.257	2.293	.107
		Total eaten, do		8.970	8.277	.693	1.754	2.079	4.164	.280
		Feces, do	17.823	3.210	2.883	.327	.463	1.266	1.037	.117
		Digested, do		5.760	5.394	.366	1.291	.813	3.127	.163
		do, percent		64.21	65.17	52.81	73.60	39.11	75.10	58.21
19, subperiod b	Steer A-24	Sweetclover silage, early bloom, pounds	16.000	3.874	3.478	.396	.823	1.255	1.264	.136
		Alfalfa hay, do	2.000	1.690	1.526	.164	.294	.542	.652	.039
		Grain mixture [2], do	4.000	3.466	3.328	.138	.670	.257	2.293	.107
		Total eaten, do		9.030	8.331	.698	1.787	2.054	4.209	.282
		Feces, do	17.073	3.106	2.780	.326	.453	1.259	.959	.109
		Digested, do		5.924	5.552	.372	1.334	.795	3.250	.173
		do, percent		65.60	66.63	53.30	74.65	38.70	77.22	61.35
19, subperiod a	Steer B-24	Sweetclover silage, early bloom, pounds	16.000	3.814	3.423	.391	.790	1.281	1.219	.134
		Alfalfa hay, do	2.000	1.690	1.526	.164	.294	.542	.652	.039
		Grain mixture [2], do	4.000	3.466	3.328	.138	.670	.257	2.293	.107
		Total eaten, do		8.970	8.277	.693	1.754	2.079	4.164	.280
		Feces, do	14.818	3.069	2.737	.332	.467	1.247	.923	.100
		Digested, do		5.901	5.540	.361	1.287	.832	3.241	.180
		do, percent		65.79	66.93	52.09	73.38	40.02	77.83	64.29
19, subperiod b	Steer B-24	Sweetclover silage, early bloom, pounds	16.000	3.874	3.478	.396	.823	1.255	1.264	.136
		Alfalfa hay, do	2.000	1.690	1.526	.164	.294	.542	.652	.039
		Grain mixture [2], do	4.000	3.466	3.328	.138	.670	.257	2.293	.107
		Total eaten, do		9.030	8.332	.698	1.787	2.054	4.209	.282
		Feces, do	15.418	3.031	2.723	.308	.448	1.214	.961	.100
		Digested, do		5.999	5.609	.390	1.339	.840	3.248	.182
		do, percent		66.43	67.32	55.87	74.93	40.90	77.17	64.54
19, subperiod a	Steer C-24	Sweetclover silage, early bloom, pounds	16.000	3.814	3.423	.391	.790	1.280	1.219	.134
		Alfalfa hay, do	2.000	1.690	1.526	.164	.294	.542	.652	.039
		Grain mixture [2], do	4.000	3.466	3.328	.138	.670	.257	2.293	.107
		Total eaten, do		8.970	8.277	.693	1.754	2.079	4.164	.280
		Feces, do	15.276	3.006	2.682	.324	.421	1.211	.945	.105
		Digested, do		5.964	5.595	.369	1.333	.868	3.219	.175
		do, percent		66.49	67.60	53.25	76.00	41.75	77.31	62.50

See footnotes at end of table.

TABLE 5.—*Digestibility of mixed rations: Sweetclover, sunflower, and corn silages, alfalfa hay, and concentrates*—Continued

Trial No.	Animal	Feeds and other items	Original weight	Dry matter	Organic matter	Ash	Crude protein	Crude fiber	N-free extract	Ether extract
19, subperiod b	Steer C-24	Sweetclover silage, early bloom ..... pounds	16.000	3.874	3.478	0.396	0.823	1.255	1.264	0.136
		Alfalfa hay ..... do	2.000	1.690	1.526	.164	.294	.542	.652	.039
		Grain mixture [2] ..... do	4.000	3.466	3.328	.138	.670	.257	2.293	.107
		Total eaten		9.030	8.332	.698	1.787	2.054	4.209	.282
		Feces ..... do	14.814	2.886	2.591	.295	.397	1.194	.899	.101
		Digested ..... do		6.144	5.741	.403	1.390	.860	3.310	.181
		..... do ..... percent		68.04	68.90	57.74	77.78	41.87	7.864	64.18
19, subperiod a	Steer D-24	Sweetclover silage, early bloom ..... pounds	16.000	3.814	3.423	.391	.790	1.280	1.219	.134
		Alfalfa hay ..... do	2.000	1.690	1.526	.164	.294	.542	.652	.039
		Grain mixture [2] ..... do	4.000	3.466	3.327	.138	.670	.257	2.293	.107
		Total eaten ..... do		8.970	8.278	.693	1.754	2.079	4.164	.280
		Feces ..... do	15.453	2.959	2.636	.323	.415	1.210	.913	.098
		Digested ..... do		6.011	5.641	.370	1.339	.869	3.251	.182
		..... do ..... percent		67.01	68.15	53.39	76.34	41.80	78.07	65.00
19, subperiod b	do	Sweetclover silage, early bloom ..... pounds	16.000	3.874	3.478	.396	.823	1.255	1.264	.136
		Alfalfa hay ..... do	2.000	1.690	1.526	.164	.294	.542	.652	.039
		Grain mixture [2] ..... do	4.000	3.466	3.328	.138	.670	.257	2.293	.107
		Total eaten ..... do		9.030	8.332	.698	1.787	2.054	4.209	.282
		Feces ..... do	15.524	3.033	2.715	.318	.416	1.292	.905	.102
		Digested ..... do		5.997	5.617	.380	1.371	.762	3.304	.180
		..... do ..... percent		66.41	67.41	54.44	76.72	37.10	78.50	63.83
12	Steer A-22	Sunflower silage ..... pounds	14.000	3.647	3.100	.547	.299	1.391	1.333	.077
		Alfalfa hay ..... do	5.000	4.301	3.860	.441	.696	1.534	1.568	.062
		Linseed meal ..... do	2.000	1.761	1.642	.119	.702	.166	.647	.127
		Total ..... do		9.709	8.602	1.107	1.697	3.091	3.548	.266
		Feces ..... do	16.092	3.988	3.561	.427	.452	1.808	1.233	.068
		Digested ..... do		5.721	5.041	.680	1.245	1.283	2.315	.198
		..... do ..... percent		58.92	58.60	61.43	73.36	41.51	65.25	74.44

		Sunflower silage ---- pounds	14.000	3.647	3.100	.547	.299	1.391	1.333	.077
		Alfalfa hay ---- do	5.000	4.301	3.860	.441	.696	1.534	1.568	.062
		Linseed meal ---- do	2.000	1.761	1.642	.119	.702	.166	.647	.127
12	Steer B-22	Total ---- do		9.709	8.602	1.107	1.697	3.091	3.548	.266
		Feces ---- do	20.285	3.970	3.529	.441	.480	1.772	1.205	.073
		Digested ---- do		5.739	5.073	.666	1.217	1.319	2.343	.193
		---- do ---- percent		59.11	58.97	60.16	71.71	42.67	66.04	72.56
		Corn silage ---- pounds	20.000	6.094	5.662	.432	.505	1.370	3.617	.169
		Alfalfa hay ---- do	5.000	4.320	3.912	.408	.647	1.686	1.519	.060
		Linseed meal ---- do	2.000	1.770	1.650	.121	.702	.160	.666	.121
14	Steer A-22	Total ---- do		12.184	11.224	.961	1.854	3.216	5.802	.350
		Feed residue [3] ---- do	[3] .612	.565	.513	.051	.139	.149	.209	.017
		Total eaten ---- do		11.619	10.711	.910	1.715	3.067	5.593	.333
		Feces ---- do	18.310	3.924	3.423	.501	.567	1.457	1.313	.086
		Digested ---- do		7.695	7.288	.409	1.148	1.610	4.280	.247
		---- do ---- percent		66.23	68.04	44.95	66.94	52.49	76.52	74.17
		Corn silage ---- pounds	20.000	6.094	5.662	.432	.505	1.370	3.617	.169
		Alfalfa hay ---- do	5.000	4.320	3.912	.408	.647	1.686	1.519	.060
		Linseed meal ---- do	2.000	1.770	1.650	.121	.702	.160	.666	.121
		Total ---- do		12.184	11.224	.961	1.854	3.216	5.802	.350
14	Steer B-22	Feed residue [3] ---- do	[3] 1.838	1.698	1.550	.148	.286	.649	.583	.032
		Feed residue [4] ---- do	[4] .032	.029	.027	.002	.003	.008	.015	.001
		Total residue ---- do		1.727	1.577	.150	.289	.657	.598	.033
		Total eaten ---- do		10.457	9.647	.811	1.565	2.559	5.204	.317
		Feces ---- do	21.470	3.860	3.311	.549	.528	1.255	1.452	.078
		Digested ---- do		6.597	6.336	.262	1.037	1.304	3.752	.239
		---- do ---- percent		63.09	65.68	32.31	66.26	50.96	72.12	75.39
		Alfalfa hay ---- pounds	7.000	5.991	5.368	.623	.999	2.134	2.160	.075
		Linseed meal ---- do	2.000	1.771	1.653	.118	.702	.171	.658	.122
17	Steer C-22	Total ---- do		7.762	7.021	.741	1.701	2.305	2.818	.197
		Feces ---- do	17.695	3.088	2.671	.417	.405	1.363	.826	.077
		Digested ---- do		4.674	4.350	.324	1.296	.942	1.992	.120
		---- do ---- percent		60.22	61.96	43.72	76.19	40.87	70.69	60.91

See footnotes at end of table.

TABLE 5.—*Digestibility of mixed rations: Sweetclover, sunflower, and corn silages, alfalfa hay, and concentrates*—Continued

Trial No.	Animal	Feeds and other items	Original weight	Dry matter	Organic matter	Ash	Crude protein	Crude fiber	N-free extract	Ether extract
17	Steer D-22	Alfalfa hay, pounds	7.000	5.991	5.368	0.623	0.999	2.134	2.160	0.075
		Linseed meal, do	2.000	1.771	1.653	.118	.703	.171	.658	.122
		Total, do		7.762	7.021	.741	1.701	2.305	2.818	.197
		Feces, do	25.252	3.280	2.918	.362	.446	1.484	.903	.085
		Digested, do		4.482	4.103	.379	1.255	.821	1.915	.112
		Digested, percent		57.74	58.44	51.15	73.78	35.62	67.96	56.85
21, subperiod a	Steer A-24	Alfalfa hay, pounds	8.000	6.827	6.179	.648	1.146	2.315	2.593	.125
		Wild oats, do	8.000	7.046	6.735	.311	1.937	.092	4.282	.424
		Total, do	16.000	13.873	12.914	.959	2.083	3.407	6.875	.549
		Residue, do	1.630	1.503	1.373	.130	.254	.424	.651	.044
		Total eaten, do	14.370	12.370	11.541	.829	1.829	2.983	6.225	.505
		Feces, do	24.000	4.932	4.472	.460	.527	2.010	1.837	.098
		Digested, do		7.438	7.069	.369	1.302	.973	4.388	.407
		Digested, percent		60.13	61.25	44.51	71.19	32.62	70.49	80.59
21, subperiod b	Steer A-24	Alfalfa hay, pounds	8.000	6.827	6.179	.648	1.146	2.315	2.593	.125
		Wild oats, do	8.000	7.046	6.735	.311	.937	1.092	4.282	.424
		Total, do	16.000	13.873	12.914	.959	2.083	3.407	6.875	.549
		Residue, do	1.640	1.480	1.348	.132	.250	.434	.623	.041
		Total eaten, do	14.360	12.393	11.566	.827	1.833	2.973	6.252	.508
		Feces, do	22.773	4.782	4.347	.435	.511	1.943	1.784	.109
		Digested, do		7.611	7.218	.392	1.322	1.030	4.468	.399
		Digested, percent		61.41	62.41	47.40	72.12	34.65	71.47	78.54
21, subperiod a	Steer B-24	Alfalfa hay, pounds	8.000	6.827	6.179	.648	1.146	2.315	2.593	.125
		Wild oats, do	8.000	7.046	6.735	.311	.937	1.092	4.282	.424
		Total, do	16.000	13.873	12.914	.959	2.083	3.407	6.875	.549
		Feces, do	25.370	5.673	5.161	.512	.609	2.347	2.094	.111
		Digested, do		8.200	7.753	.447	1.474	1.060	4.781	.438
		Digested, percent		59.11	60.04	46.61	70.76	31.11	69.54	79.78

21, subperiod b	Steer B–24	Alfalfa hay, pounds	8.000	6.827	6.179	.648	1.146	2.315	2.593	.125
		Wild oats, do	8.000	7.046	6.735	.311	.937	1.092	4.282	.424
		Total, do	16.000	13.873	12.914	.959	2.083	3.407	6.875	.549
		Feces, do	24.815	5.683	5.163	.520	.611	2.394	2.040	.117
		Digested, do		8.190	7.751	.439	1.472	1.013	4.835	.432
		do, percent		59.04	60.02	45.78	70.67	29.73	70.33	78.69

[1] Sweetclover silage residue, air-dried weight.
[2] Ground barley, 3 parts; linseed meal, 1 part.
[3] Alfalfa and linseed-meal residues.
[4] Corn-silage residue.

COEFFICIENTS OF DIGESTIBILITY, INDIRECTLY COMPUTED

Since the ratio of alfalfa hay to linseed meal was constant in the different trials of 1923–24, excepting trial 17, it was possible to compute the digestibility of some of the components of the mixed rations. By applying the coefficients of digestibility of the sweetclover silages obtained with steers A–22 and B–22 in trials 8 and 10 to the data of steers C–22 and D–22 in trials 9 and 11, it was possible to compute the digestibility of linseed meal and alfalfa hay when combined in the ratio of 2 to 5, respectively. The coefficients thus computed were applied to the data of steers A–22 and B–22 in trials 12 and 14 in computing, indirectly, the digestibility of the sunflower and corn silages. The digestibility of the linseed meal was also computed by applying the determined coefficients of digestibility of alfalfa and the silages to the respective trials and obtaining the digestibility of the linseed meal by difference.

Similarly, in the trials of 1924–25, the digestibility of the grain mixture consisting of 3 parts of barley and 1 part of linseed meal was computed and also the digestibility of the wild oats.

The coefficients thus computed are given in table 6.

TABLE 6.—*Coefficients of digestibility of feed constituents, indirectly computed*

Trial No.	Animal No.	Feeds	Dry matter	Organic matter	Crude protein	Crude fiber	N-free extract	Ether extract
			Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
9	C–22	Alfalfa 5 parts and linseed meal 2 parts	60.6	61.7	74.6	38.6	70.5	60.5
	D–22		61.5	62.8	76.8	41.1	69.8	66.9
		Average	61.1	62.3	75.7	39.9	70.2	63.7
11	C–22	Alfalfa 5 parts and linseed meal 2 parts	61.9	63.4	77.2	40.9	70.9	72.0
	D–22		61.6	62.5	77.0	39.1	69.9	75.1
		Average	61.8	63.0	77.1	40.0	70.4	73.6
12	A–22	Sunflower silage	54.9	51.5	59.4	43.4	56.9	88.5
	B–22	do	55.4	52.5	50.1	46.0	59.0	82.8
		Average	55.2	52.0	54.8	44.7	58.0	85.7
14	A–22	Corn silage	70.6	72.9	44.2	67.9	80.0	79.3
	B–22	do	55.2	67.9	45.9	61.4	72.8	82.3
		Average	62.9	70.4	45.1	64.7	76.4	80.8
9	C–22	Linseed meal	69.1	68.2	79.7	−122.0	74.4	88.3
	D–22	do	72.1	72.1	84.0	2.5	72.0	97.9
		Average	70.6	70.2	81.9	−59.8	73.2	93.1
11	C–22	Linseed meal	73.4	73.9	84.9	−100.1	75.8	105.9
	D–22	do	72.3	71.0	84.4	−118.3	72.5	110.6
		Average	72.9	72.5	84.7	−109.2	74.2	108.3
12	A–22	Linseed meal	71.5	70.6	85.3	−116.3	71.7	100.6
	B–22	do	72.6	72.6	81.3	−100.2	76.0	97.1
		Average	72.1	71.6	83.3	−108.3	73.9	98.9
14	A–22	Linseed meal	93.5	93.7	78.4	78.7	118.2	98.3
	B–22	do	64.7	70.5	77.6	34.4	71.9	91.0
		Average	79.1	82.1	78.0	56.6	95.1	94.7

TABLE 6.—*Coefficients of digestibility of feed constituents, indirectly computed*—Con.

Trial No.	Animal No.	Feeds	Dry matter	Organic matter	Crude protein	Crude fiber	N-free extract	Ether extract
			Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
17	C–22	Linseed meal	71.1	72.3	85.9	−116.9	76.8	94.3
	D–22	do	60.3	57.3	80.0	−187.9	65.1	87.7
		Average	65.7	64.8	83.0	−152.4	71.0	91.0
		Linseed meal [1]	72.1	72.2	82.2	−74.6	77.5	97.2
		do [2]	70.3	69.8	83.2	−107.4	73.1	97.8
19	A–24	Barley 3 parts and linseed meal 1 part	69.9	71.4	69.3	25.3	78.7	60.3
	B–24	do	73.0	74.4	69.4	37.8	81.1	72.4
19	C–24	do	76.0	77.2	76.7	48.6	82.0	69.6
	D–24	do	74.6	76.0	75.7	29.8	82.6	72.4
		Average	73.4	74.8	72.8	35.4	81.1	68.7
21	A–24	Wild oats	62.7	64.0	73.6	22.3	69.6	93.5
	B–24	do	59.6	60.9	71.3	11.5	50.6	95.0
		Average	61.2	62.5	72.5	16.9	60.1	94.3

[1] Average all trials.
[2] Average omitting trial No. 14.

Reference to table 6 shows that the average coefficients for alfalfa hay and linseed meal combined, as computed in trials 9 and 11, agree fairly with each other except for ether extract where the difference between the averages of the two trials is 10 percent. On the whole, these coefficients for alfalfa 5 parts and linseed meal 2 parts are, as would be expected, slightly higher but similar to the coefficients directly determined in trial 17 for alfalfa 7 parts and linseed meal 2 parts, table 7.

The indirectly computed coefficients for sunflower silage, trial 12, steers A–22 and B–22, agree within 3 percent of each other except for protein where the difference is 9.3 and for ether extract where the difference is 5.7. Compared to the single direct determination with steer C–22, trial 13, the indirectly computed coefficients are higher by 5.2 for dry matter, 3.5 for organic matter, 10.9 for crude protein, 5.0 for crude fiber, 0.8 for nitrogen-free extract and 13.2 for ether extract. Steer C–22 ate only enough sunflower silage to furnish 0.4 as much digestible crude protein and less than half as much digestible nutrients as are needed for maintenance on the basis of the feeding standards for maintenance proposed by Brody, Procter, and Ashworth (*2*). Steers A–22 and B–22, trial 12, consumed adequate amounts of protein but their rations were a little low in digestible nutrients. The low protein consumption by steer C–22 may account in part for the low digestibility of the crude protein by this steer.

The indirectly computed coefficients of digestibility for corn silage, steers A–22 and B–22, trial 14, differ by 15.4 for dry matter, 5.0 for organic matter, 1.7 for crude protein, 6.5 for crude fiber, 7.2 for nitrogen-free extract and 3.0 for ether extract. The wide difference in the coefficients for dry matter may be due in part to unavoidable errors in apportioning the alfalfa, linseed meal, and corn silage in the feed residues, especially with steer B–22. Except for the dry matter, the

digestion coefficients show about the same degree of variation between those computed indirectly, those determined directly, and between the averages of the two.

The computed digestion coefficients for linseed meal are rather unsatisfactory, particularly the coefficients for crude fiber which were negative by 100 or more in 7 out of 10 trials. If the results of trial 14 are omitted, the agreement among the coefficients for dry matter, organic matter, crude protein, nitrogen-free extract, and, to a lesser degree, for the ether extract, is fairly satisfactory. The relatively small amounts of linseed meal in the rations and the low fiber content of linseed meal tend to make the results somewhat uncertain. With trial 14 omitted, the average digestion coefficients for linseed meal are 83.2 for crude protein, —107.4 for crude fiber, 73.1 for nitrogen-free extract and 97.8 for ether extract.

SUMMARY OF DIGESTION COEFFICIENTS

The average coefficients of digestibility for the different feeds and mixed rations are summarized in table 7.

Reference to table 7 shows that the sweetclover-oat straw silage was distinctly lower in digestibility of all nutrients except crude fiber than the sweetclover silage from the same crop (1922). Mixing 5.5 parts of freshly cut sweetclover with 1 part of dry oat straw gave a silage mixture in which only 53.6 percent of the dry matter was sweetclover. Compared to the sweetclover silage the inclusion of the straw reduced the coefficients of digestibility of dry matter from 56.9 to 33.9; organic matter from 56.6 to 35.1; crude protein from 76.8 to 51.5; nitrogen-free extract from 58.0 to 12.8; ether extract from 62.8 to 55.6, while the coefficients for the crude fiber remained virtually identical.

A comparison of the sweetclover silage of 1922, trial 2, with the first and second cuttings of sweetclover hay, the same year, trials 5 and 6, shows that the coefficients are essentially alike, except for the notably lower digestibility of the ether extract in the hays. Similar relationships are noted in comparing the digestibility of the sweet clover silages and alfalfa hays.

The coefficients from a single trial on the sweetclover stems are about the same for crude fiber and ether extract as those for the second cutting of sweetclover hay cut in early bloom, but are lower for all the other nutrients.

The sweetclover silage, full bloom, trial 10, with its higher fiber content, gave slightly higher coefficients of digestibility for dry matter, organic matter, crude protein, and crude fiber, but lower coefficients for nitrogen-free extract and ether extract than the silage from the sweetclover cut in early bloom, trial 8. The sweetclover silage of 1924, trial 20, gave consistently higher coefficients for all nutrients, except for crude fiber in trial 10, than the silages of the two preceding years. This silage was superior in appearance and palatability to the silages fed in the preceding years. The sweetclover silages were not relished at first by either the sheep or steers, but after acquiring a taste for them they ate them readily.

TABLE 7.—*Summary of digestion-coefficient data for the various feeds*

Feeds	Trial No.	Experimental animals	Individual trials	Year of experiment	Dry matter	Organic matter	Crude protein	Crude fiber	N-free extract	Ether extract
			Number		*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Sweetclover-oat straw silage	1	Sheep	2	1922–23	33.9	35.1	51.5	41.7	12.8	55.6
Sweetclover silage, bud stage	2	do	3	1922–23	56.9	56.6	76.8	41.8	58.0	62.8
Sweetclover silage, early bloom	8	Steers	2	1923–24	58.7	58.6	69.7	38.2	69.0	58.7
Sweetclover silage, full bloom	10	do	2	1923–24	60.0	59.7	73.3	43.4	67.3	48.6
Sweetclover silage, early bloom	20	do	[1] 4	1924–25	63.7	64.0	79.6	42.8	73.3	66.3
Sweetclover hay, first cutting, early bloom, ground	5	Sheep	1	1922–23	56.8	55.0	75.3	41.5	61.9	37.4
Sweetclover hay, second cutting, early bloom, ground	6	do	2	1922–23	57.5	57.5	76.4	39.7	67.0	28.5
Sweetclover, chiefly stems, ground	7	do	1	1922–23	45.7	45.1	68.1	39.3	48.3	29.6
Alfalfa hay	16	Steers	2	1923–24	57.0	58.8	69.4	45.5	68.8	7.2
	18	do	4	1924–25	57.3	58.3	70.2	36.1	72.9	35.1
	22	do	2	1924–25	58.5	59.1	70.3	39.4	73.5	25.9
Sunflower silage	3	Sheep	3	1922–23	52.5	53.2	51.1	42.0	59.8	65.9
	13	Steers	1	1923–24	50.0	48.5	43.9	39.7	57.2	72.5
	12	do	[2] 2	1923–24	55.2	52.0	54.8	44.7	58.0	85.7
Average		do	3	1923–24	53.5	50.8	51.2	43.0	57.7	81.3
Corn silage	4	Sheep	3	1922–23	73.3	76.3	69.1	70.4	79.6	82.0
	15	Steers	2	1923–24	65.4	67.4	50.1	57.7	72.9	80.8
	14	do	[2] 2	1923–24	62.9	70.4	45.1	64.7	76.4	80.8
Sweetclover silage, early bloom, alfalfa hay, and linseed meal	9	do	2	1923–24	59.7	60.2	72.7	38.8	69.6	61.1
Sweetclover silage, full bloom, alfalfa hay, and linseed meal	11	do	2	1923–24	60.9	61.5	75.2	41.6	69.3	63.3
Sweetclover silage, alfalfa hay, and grain mixture	19	do	[1] 4	1924–25	66.3	67.3	75.4	40.2	77.5	63.0
Alfalfa hay, 5 parts; linseed meal, 2 parts	9 and 11.	do	4	1923–24	61.5	62.7	71.4	39.0	70.3	73.7
Alfalfa hay, 7 parts; linseed meal, 2 parts	17	do	2	1923–24	59.0	60.2	75.0	38.2	69.3	58.9
Alfalfa hay and wild oats	21	do	[3] 2	1924–25	59.9	60.9	71.2	31.9	70.4	79.4
Sunflower silage, alfalfa hay, and linseed meal	12	do	2	1923–24	59.0	58.8	72.5	42.1	65.6	73.5
Corn silage, alfalfa hay, and linseed meal	14	do	2	1923–24	64.7	66.9	66.6	51.8	74.4	74.8
Linseed meal		do	8	1923–24	70.3	69.8	83.2	−107.4	73.1	97.8
Linseed meal, Morrison [4]		do	9				87.0	59.0	82.0	92.0
Barley, 3 parts; linseed meal, 1 part	19	do	4	1924–25	78.1	79.2	76.8	55.4	83.6	74.4
Wild oats	21	do	[3] 2	1924–25	61.2	62.5	72.5	16.9	60.1	94.3
Oats, common, Morrison [4]			19				78.0	38.0	81.0	88.0

[1] The trials covered 18 days, with 9-day subperiods.
[2] Digestibility indirectly computed.
[3] This 20-day period was divided into subperiods of 10 days each.
[4] Morrison (*8*).

The sunflower silages were unpalatable and both sheep and steers refused to eat them when first offered. One steer would not eat them at all and the other ate less than a maintenance ration. Compared to corn silage the sunflower silage gave notably lower digestion coefficients for all nutrients except crude protein.

The corn silage was palatable and gave distinctly higher coefficients than the sweetclover or sunflower silages except for crude protein.

For comparison the average coefficients for linseed meal from nine trials as given by Morrison (*8*) are included in table 7. Morrison's averages are higher for protein, crude fiber, and nitrogen-free extract, but lower for ether extract than were those of the eight linseed-meal tests recorded.

The wild oats fed in these trials were heavier than the usual run, weighing 37.5 pounds to the bushel. Compared to Morrison's (*8*) average coefficients for common or cultivated oats, they are somewhat lower except for ether extract. Additional trials are needed before the data can be considered representative of wild oats.

APPARENT AND "TRUE" DIGESTIBILITY OF PROTEIN

Both the sunflower and corn silages had wide nutritive ratios and low apparent digestibility of the protein. Reference to table 8 shows that the low digestibility of the crude protein is more apparent than real. When correction is made for the metabolic nitrogen by the methods of either Mitchell (*7*) or Titus (*9*), the "true" digestibility does not vary greatly in the different feeds.

TABLE 8.—*Apparent and "true" digestibility of protein in silages and alfalfa hay, crops of 1923*

Silage or hay	Trial No.	Digestibility of protein			Nutritive ratio 1:—
		Apparent	True		
			Mitchell	Titus [1]	
		Percent	*Percent*	*Percent*	
Sweetclover silage	8	69.7	86.7	85.5	3.4
Sunflower silage	10	73.3	87.9	88.2	2.5
Corn silage	8	48.5	82.0	82.7	11.0
Alfalfa hay	15	50.1	87.7	87.7	41.8
	17	69.4	88.1	89.1	3.6

[1] Formula 2.

DIGESTIBLE NUTRIENTS IN FEEDS

The percentages of dry matter in the feeds and the digestible crude protein and total digestible nutrients in the feeds, both as fed and on a dry-matter basis, are given in table 9.

TABLE 9.—*Percentages of dry matter, digestible crude protein, and total digestible nutrients in feeds*

Feeds	Year of experiment	Trial No.	Experimental animals	Individual trials	As fed			In dry matter	
					Dry matter	Digestible crude protein	Total digestible nutrients	Digestible crude protein	Total digestible nutrients
				Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Sweetclover-oat-straw silage	1922–23	1	Sheep	2	21.97	1.6	7.4	7.3	33.7
Sweetclover silage, bud stage	1922–23	2	do	3	34.78	5.8	18.6	16.7	53.5
Sweetclover silage, early bloom	1923–24	8	Steers	2	33.47	4.3	18.6	12.8	55.6
Sweetclover silage, full bloom	1923–24	10	do	2	32.66	5.1	18.1	15.6	55.4
Sweetclover silage, early bloom	1924–25	20	do	4	24.70	4.4	15.0	17.9	60.7
Average				11	30.49	4.9	17.2	16.2	56.8
Sweetclover hay, first cutting, ground	1922–23	5	Sheep	1	82.46	10.1	41.3	12.3	50.1
Sweetclover hay, second cutting, ground	1922–23	6	do	2	82.58	11.6	44.5	14.0	53.9
Average				3	82.54	11.1	43.4	13.4	52.6
Sweetclover, chiefly stems, ground.	1922–23	7	Sheep	1	85.33	5.5	36.4	6.4	42.7
Alfalfa hay	1923–24	16	Steers	2	84.59	9.8	44.7	11.6	52.8
Alfalfa hay	1924–25	18	do	4	84.81	10.2	45.4	12.1	53.5
Alfalfa hay	1924–25	22	do	2	85.34	10.1	46.2	11.8	54.1
Average				8	84.89	10.1	45.4	11.9	53.5
Sunflower silage	1922–23	3	Sheep	3	26.18	1.4	13.0	5.3	49.7
Sunflower silage	1923–24	12 and 13	Steers	3	26.05	1.1	11.9	4.2	45.7
Average				6	26.12	1.3	12.3	4.8	47.7
Corn silage	1922–23	4	Sheep	3	24.35	1.7	17.8	7.0	73.1
Corn silage	1923–24	15	Steers	2	30.47	1.3	20.0	4.3	65.6
Corn silage	1923–24	14	do	2	30.47	1.1	21.0	3.7	68.7
Average				7	27.85	1.4	19.3	5.3	69.7
Linseed meal [1]	1923–24		Steers	8	88.49	29.1	67.1	32.9	75.8
Linseed meal [2]					91.3	30.6	78.2	33.5	85.7
Wild oats	1924–25	21	do	2	88.07	8.5	54.2	9.6	61.6
Oats, common [2]					91.1	9.4	71.5	10.3	78.5

[1] Trials 9, 11, 12, and 17.
[2] Morrison (8).

From table 9 it is clear that the sweetclover-oat straw silage, trial 1, was decidedly lower in digestible protein and total digestible nutrients than the sweetclover silage made from the same crop, trial 2. Also, the table indicates clearly the importance of considering the dry-matter content of silages in making comparisons of nutritive values. The sweetclover silages fed in trials 2, 8, and 10 had approximately the same dry-matter content, digestible protein, and total digestible nutrients on the fresh basis. The silage fed in trial 20 contained 8 to 10 percent less dry matter and somewhat less total digestible nutrients than the three preceding silages, but, when compared on a dry-matter basis, this silage excelled the others in digestible protein and total digestible nutrients. Similarly, the corn silage fed in trial 4, because of its high water content, contained less digestible protein and total digestible nutrients than the corn silage fed in trials 14 and 15, but, on a dry-matter basis, it was really superior.

When compared on a dry-matter basis, the sweetclover silages and hays are similar to the alfalfa hays in nutritive values, but the sweetclover silages are, in general, a little higher in digestible protein and total digestible nutrients. Compared to the corn silages, the sweetclover silages are much higher in digestible crude protein, but lower in total digestible nutrients.

The sunflower silages contained about the same amounts of digestible crude protein as the corn silages, but in total digestible nutrients they were much lower than the corn, and considerably lower than the sweetclover silages. Of the three silages tested, the sunflowers were the least palatable and the lowest in nutritive value.

The corn silages used in these trials were made from Mercer flint corn. At corresponding stages of maturity and yields they have the same composition and nutritive values as silages from dent corn.

The mixing of oat straw with the sweetclover to reduce the moisture content of the silage and aid in its preservation proved unsatisfactory. The silage had a very strong odor and was less palatable than the straight sweetclover silage.

During a period of several years, sweetclover silage of good quality has been made at this station by allowing the sweetclover to wilt until it had a dry-matter content ranging from about 25 to 35 percent before ensiling it. By packing it thoroughly, the spoilage nas not been excessive and no evidences of so-called sweetclover poisoning have appeared. The digestion trials here reported indicate that when allowance is made for differences in water content, the sweetclover silages have essentially the same nutritive values as the green sweetclover or the cured hay, so far as digestibility is concerned. Unless weather conditions are ideal it is very difficult to make sweetclover hay which is free from mold. With unfavorable weather conditions for haymaking, the silo provides a means for storing the sweetclover in a safe and usable form. Sweetclover silage is not as palatable as corn silage; in fact it is usually necessary to reduce other feeds at first to induce stock to eat the silage. However, after becoming accustomed to it, they will eat it readily. In considering the value of sweetclover silage, its relatively high protein content is not to be ignored.

Our experience with the Russian sunflower indicates that it has no particular merit as a silage crop in this section. The sunflower silage is less palatable than the sweetclover silage.

The alfalfa hays fed in these trials represent good alfalfa hay in digestible crude protein and total digestible nutrients.

The digestible crude protein in the linseed meal on a dry basis is nearly the same as the value given by Morrison (*8*), but the total digestible nutrients are about 10 percent lower.

Since no values for wild oats have come to the authors' attention, the values given by Morrison for common oats are included in table 9 for comparison. Compared on a dry basis the wild oats contained nearly as much digestible crude protein as the common oats, but approximately 17 percent less of total digestible nutrients.

AGE, LIVE WEIGHTS, AND DAILY NITROGEN BALANCES OF STEERS

The actual age of the steers was not known, and the ages given in table 10 are only approximate. The average live weights were computed from three to five weights on successive days, immediately

before and after the trials. As the feeds and excreta were weighed on scales graduated in pounds and decimals of pounds, the nitrogen balances are also expressed decimally in pounds. The nitrogen balances were determined primarily for the energy data.

TABLE 10.—*Age of steers, live weights, and daily nitrogen balances*

Steer	Approximate age	Average live weight	Trial No.	Feeds	Nitrogen			
					Feed	Feces	Urine	Balance
	Months	*Pounds*			*Pounds*	*Pounds*	*Pounds*	*Pounds*
A-22	21	902	8	Sweet clover silage, early bloom	0.3882	0.1153	0.2663	+0.0066
B-22	21	866			.3923	.1227	.2572	+.0124
C-22	20	828	9	Sweetclover silage, early bloom alfalfa and linseed meal.	.4583	.1280	.3066	+.0237
D-22	20	823			.4331	.1158	.2953	+.0220
A-22	22	912	10	Sweetclover silage, full bloom	.4256	.1075	.2309	+.0872
B-22	22	884			.4464	.1256	.3516	+.0308
C-22	21	833	11	Sweetclover silage, full bloom alfalfa and linseed meal.	.3768	.0936	.3057	−.0225
D-22	21	833			.4125	.1021	.3238	−.0134
A-22	23	884	12	Sunflower silage, alfalfa, and linseed meal.	.2716	.0723	.1897	+.0096
B-22	23	861			.2716	.0767	.1925	+.0024
C-22	22	792	13	Sunflower silage	.0820	.0460	.0547	−0187
A-22	24	918	14	Corn silage, alfalfa, and linseed meal	.2744	.0908	.1633	+.0203
B-22	24	877			.2504	.0844	.1567	+.0093
C-22	23	788	15	Corn silage	.1200	.0543	.0366	+.0291
D-22	23	807			.1212	.0663	.0383	+.0166
A-22	25	882	16	Alfalfa hay	.2513	.0765	.1982	−.0234
B-22	25	874			.2513	.0773	.1741	−.0001
C-22	24	784	17	Alfalfa hay and linseed meal	.2723	.0648	.1952	+.0123
D-22	24	801			.2723	.0714	.2078	−.0069
A-24	7.5	467	18	Alfalfa hay	.2329	.0674	.1391	+.0264
B-24	7.5	456			.2329	.0704	.1605	+.0020
C-24	7.5	470			.2329	.0694	.1378	+.0257
D-24	7.5	428			.2096	.0630	.1401	+.0065
A-24	9.5	498	19	Sweetclover, silage, alfalfa, and grain	.2833	.0733	.1782	+.0318
B-24	9.5	504			.2833	.0733	.1743	+.0357
C-24	9.5	528			.2833	.0655	.1771	+.0407
D-24	9.5	466			.2833	.0665	.1763	+.0405
A-24	10.5	502	20	Sweetclover silage	.2308	.0469	.1880	−.0041
B-24	10.5	513			.2308	.0472	.1857	−.0021
C-24	10.5	530			.2485	.0512	.2040	−.0067
D-24	10.5	484			.2485	.0507	.2057	−.0079
A-24	11.5	571	21	Alfalfa hay and wild oats	.2932	.0831	.1615	+.0486
B-24	11.5	579			.3334	.0977	.1864	+.0493
C-24	11.5	583	22	Alfalfa hay	.2891	.0882	.1865	+.0234
D-24	11.5	540			.2981	.0889	.1855	+.0237

METABOLIZABLE ENERGY IN FEEDS AND RATIONS

The gross energy in the feed, feces, and urine was determined directly by means of a Parr adiabatic oxygen-bomb calorimeter. According to Armsby (*1*), 100 g of carbohydrates digested by ruminants produce 4.5 g of methane, with an energy value of 60.1 calories which is equivalent to 272.6 calories or 0.2726 therm per pound. The latter value was used in computing the energy value of the methane. The energy in the urine was corrected for gain or loss of protein by the steers by use of Rubner's value for protein nitrogen as employed by Armsby. The energy data on all the rations fed to steers are given in table 11.

Armsby (*1*) has shown that there is a rather striking uniformity in the metabolizable energy per unit of digestible organic matter in feeds and rations. He summarized the values found by various investigators and found that in 73 individual trials with cattle the metabolizable energy per pound of digestible organic matter in roughages ranged from 1.50 to 1.70 therms with an average of 1.60 therms. In 31 trials the values for concentrates ranged from 1.72 to 2.20 therms with an average of 1.83 therms, and in 76 trials the values for mixed rations ranged from 1.58 to 1.87 therms with an average of 1.66 therms.

TABLE 11.—*Gross energy of rations, losses in excreta, and metabolizable energy*

Trial No.	Steer No.	Feeds	Energy in rations and excreta									Metabolizable energy—	
			Gross energy of feed	Losses			Metabolizable	Percentage losses of energy			Metabolizable	Per pound of digestible organic matter	Per pound of total digestible nutrients
				In feces	In urine	As methane		In feces	In urine	As methane			
			Therms	*Therms*	*Therms*	*Therms*	*Therms*	*Percent*	*Percent*	*Percent*	*Percent*	*Therms*	*Therms*
8	A–22	Sweetclover silage, early bloom	28.3683	11.895	2.332	1.412	12.731	41.93	8.22	4.98	44.88	1.782	1.711
	B–22		28.3655	11.976	2.307	1.407	12.675	42.22	8.13	4.96	44.69	1.787	1.712
		Average						42.08	8.18	4.97	44.79	1.785	1.712
10	A–22	Sweetclover silage, full bloom	26.2439	10.318	2.725	1.298	11.903	39.32	10.38	4.94	45.36	1.718	1.664
	B–22		27.4549	11.975	2.442	1.264	11.773	43.62	8.89	4.61	42.88	1.731	1.682
		Average						41.47	9.64	4.78	44.12	1.725	1.673
20	A–24, subperiod a	Sweetclover silage, early bloom	13.6679	4.959	1.487	.652	6.570	36.28	10.88	4.77	48.07	1.771	1.681
	A–24, subperiod b		13.5029	4.894	1.370	.651	6.588	36.24	10.15	4.82	48.79	1.786	1.693
	B–24, subperiod a		13.6679	4.854	1.460	.657	6.697	35.51	10.68	4.81	49.00	1.797	1.709
	B–24, subperiod b		13.5029	4.822	1.380	.654	6.647	35.71	10.22	4.84	49.23	1.794	1.694
	C–24, subperiod a		14.7193	5.370	1.620	.703	7.026	36.48	11.01	4.78	47.73	1.758	1.666
	C–24, subperiod b		14.5934	5.378	1.563	.694	6.958	36.85	10.71	4.76	47.68	1.765	1.671
	D–24, subperiod a		14.7193	5.429	1.636	.691	6.963	36.88	11.11	4.69	47.31	1.762	1.669
	D–24, subperiod b		14.5934	5.185	1.469	.706	7.233	35.53	10.07	4.84	49.56	1.810	1.715
		Average						36.19	10.60	4.79	48.42	1.780	1.687
8	C–22	Sunflower silage	11.7719	6.414	.441	.615	4.302	54.49	3.74	5.22	36.55	1.670	1.596
15	C–22	Corn silage	18.2490	5.748	.735	1.436	10.330	31.50	4.03	7.87	56.60	1.755	1.681
	D–22		18.4339	6.821	.684	1.352	9.577	37.00	3.71	7.33	51.96	1.740	1.664
		Average						34.25	3.87	7.60	54.28	1.747	1.673
16	A–22	Alfalfa hay	18.8765	8.281	1.127	1.053	8.415	43.87	5.97	5.58	44.58	1.694	1.688
	B–22		18.8765	8.360	1.021	1.052	8.443	44.29	5.41	5.57	44.73	1.706	1.704
		Average						44.08	5.69	5.58	44.66	1.700	1.696
18	A–24	Alfalfa hay	16.9398	7.637	.960	.904	7.439	45.08	5.67	5.34	43.92	1.684	1.652
	B–24		16.9398	7.612	.959	.918	7.451	44.94	5.66	5.42	43.98	1.671	1.637
	C–24		16.9398	7.474	.938	.936	7.593	44.12	5.53	5.53	44.82	1.680	1.651
	D–24		15.2458	6.897	.877	.818	6.654	45.24	5.75	5.37	43.65	1.674	1.643
		Average						44.85	5.65	5.42	44.09	1.677	1.646

22	C–24, subperiod a	Alfalfa hay	22.4014	9.318	1.287	1.290	10.506	41.59	5.75	5.76	46.90	1.720	1.760
	C–24, subperiod b		22.4014	9.704	1.258	1.236	10.204	43.32	5.61	5.52	45.55	1.730	1.709
	D–24, subperiod a		22.4014	9.835	1.259	1.233	10.075	43.90	5.62	5.50	44.97	1.712	1.694
	D–24, subperiod b		22.4014	9.838	1.231	1.232	10.100	43.92	4.39	5.50	45.09	1.722	1.705
		Average						43.18	5.34	5.57	45.63	1.721	1.702
9	C–22	Sweetclover, early bloom, alfalfa, linseed meal	29.6747	12.219	2.242	1.458	13.755	41.18	7.56	4.91	46.35	1.791	1.715
	D–22		27.8976	11.345	2.132	1.378	13.042	40.67	7.64	4.94	46.75	1.785	1.707
		Average						40.93	7.60	4.93	46.55	1.788	1.711
11	C–22	Sweetclover silage, full bloom, alfalfa hay, linseed meal	22.1523	8.873	1.754	1.084	10.442	40.05	7.92	4.89	47.14	1.754	1.681
	D–22		23.9586	9.632	1.950	1.143	11.233	40.20	8.14	4.77	46.89	1.767	1.692
		Average						40.13	8.03	4.83	47.02	1.761	1.687
19	A–24, subperiod a	Sweetclover silage, early bloom, alfalfa hay, grain mixture [1]	18.5901	6.864	1.338	1.074	9.314	36.92	7.20	5.78	50.10	1.727	1.664
	A–24, subperiod b		18.6343	6.557	1.395	1.103	9.579	35.19	7.49	5.92	51.41	1.725	1.661
	B–24, subperiod a		18.5901	6.544	1.320	1.110	9.616	35.20	7.10	5.97	51.73	1.736	1.668
	B–24, subperiod b		18.6343	6.456	1.396	1.114	9.668	34.65	7.49	5.98	51.88	1.724	1.656
	C–24, subperiod a		18.5901	6.444	[2]	1.114	[2]	34.66	[2]	5.99	[2]	[2]	[2]
	C–24, subperiod b		18.6343	6.126	1.420	1.137	9.951	32.87	7.62	6.10	53.40	1.733	1.668
	D–24, subperiod a		18.5901	6.313	1.389	1.123	9.765	33.96	7.47	6.04	52.53	1.733	1.664
	D–24, subperiod b		18.6343	6.451	1.432	1.108	9.643	34.62	7.68	5.95	51.75	1.717	1.651
		Average						34.76	7.44	5.97	51.83	1.728	1.662
12	A–22	Sunflower silage, alfalfa hay, linseed meal	19.3085	8.165	1.079	.981	9.085	42.28	5.59	5.08	47.05	1.802	1.718
	B–22		19.3085	8.135	1.085	.998	9.090	42.13	5.62	5.17	47.08	1.792	1.710
		Average						42.21	5.61	5.13	47.07	1.797	1.714
14	A–22	Corn silage, alfalfa hay, linseed meal	23.6947	8.198	1.278	1.606	12.613	34.60	5.39	6.78	53.23	1.731	1.660
	B–22		21.3615	7.834	1.177	1.379	10.972	36.67	5.51	6.46	51.36	1.732	1.653
		Average						35.64	5.45	6.62	52.30	1.732	1.657
17	C–22	Alfalfa hay, linseed meal	15.9872	6.316	1.099	.800	7.772	39.51	6.88	5.00	48.61	1.786	1.727
	D–22		15.9872	6.946	1.027	.746	7.268	43.45	6.43	4.67	45.46	1.771	1.712
		Average						41.48	6.66	4.84	47.04	1.779	1.720
19	A–24, subperiod a	Alfalfa hay, wild oats	25.7809	10.384	1.145	1.461	12.790	40.28	4.44	5.67	49.61	1.809	1.688
	A–24, subperiod b		25.8583	9.966	1.135	1.501	13.238	38.54	4.39	5.80	51.19	1.834	1.745
	B–24, subperiod a		28.8652	11.852	1.263	1.592	14.158	41.06	4.38	5.52	49.05	1.826	1.706
	B–24, subperiod b		28.8652	11.758	1.253	1.594	14.260	40.74	4.34	5.52	49.40	1.840	1.720
		Average						40.15	4.39	5.63	49.81	1.827	1.715

[1] Ground barley, 3 parts; linseed meal, 1 part.
[2] Energy determination not made; sample lost.

The metabolizable energy per pound of digestible organic matter as given in table 11 ranges from 1.67 to 1.81 therms for the roughages and from 1.72 to 1.84 therms for the mixed rations. The average of 25 trials is 1.737 therms for the roughages and 1.767 therms for 21 trials on mixed rations. These values are a little higher than the averages found by Armsby.

No determinations of metabolizable energy on sweetclover or sunflower silages have come to the authors' attention, but in a single determination on corn silage Forbes and associates (*3*) found 1.731 therms per pound of digestible organic matter compared to the value herein given of 1.747 therms.

The metabolizable energy per pound of total digestible nutrients ranges from 1.60 to 1.72 therms for the roughages and from 1.65 to 1.75 therms for the mixed rations. The average for the roughages is 1.670 therms and for the mixed rations, 1.689 therms or an average of 1.744 therms for the 46 trials. Forbes and Kriss found a somewhat lower value, namely; 1 pound of total digestible nutrients equivalent to 1.616 therms of metabolizable energy, according to an editorial review by Kriss (*6*).

METABOLIZABLE ENERGY IN FEEDS AND FEED MIXTURES, COMPUTED INDIRECTLY

The metabolizable energy per pound of digestible organic matter was computed indirectly for some feeds and feed mixtures similarly to the computations of digestibility. The computation of the metabolizable energy of linseed meal, trial 9, steer C–22, will serve to illustrate the method employed. The data of table 5, when corrected for an air-dry sweetclover residue of 0.263 pound, equivalent to 0.73 pound of fresh silage, show that steer C–22 consumed 24.27 pounds of sweetclover silage containing 7.379 pounds of organic matter having a digestibility of 58.6 percent, trial 8 (table 7), or 4.324 pounds of digestible organic matter. Similarly, it is found that the 5 pounds of alfalfa hay eaten contained 2.226 pounds of digestible organic matter. The digestible organic matter in the whole ration was 7.682 pounds. Subtracting the organic matter of the silage and alfalfa from the total gives 1.132 pounds of digestible organic matter in the linseed meal.

The metabolizable energy per pound of digestible organic matter in the silage was determined as 1.785 therms, trial 8, and for the alfalfa hay as 1.700 therms, trial 16 (table 11). Multiplying the digestible organic matter of the silage and alfalfa by their respective values, and adding, gives 11.502 therms of metabolizable energy in the silage and alfalfa. Deducting this from 13.755 therms of metabolizable energy in the total ration (table 11) leaves 2.253 therms of metabolizable energy in the 1.132 pounds of digestible organic matter in the linseed meal eaten or 1.99 therms per pound of digestible organic matter. The results of these computations are summarized in table 12.

TABLE 12.—*Metabolizable energy per pound of digestible organic matter and per pound of total digestible nutrients in feeds and feed mixtures, computed indirectly*

Feeds	Individual trials	Metabolizable energy			
		Per pound of digestible organic matter			Per pound of total digestible nutrients
		Maximum	Minimum	Average	
	Number	*Therms*	*Therms*	*Therms*	*Therms*
Linseed meal, all trials	10	2.19	1.70	1.97	1.72
Linseed meal, omitting trial 14 [1]	8	2.19	1.93	2.03	1.74
Barley 3 parts; linseed meal 1 part	7	1.72	1.68	1.70	1.62
Wild oats	2	1.95	1.88	1.92	1.86
Alfalfa 5 parts; linseed meal 2 parts	4	1.80	1.77	1.79	1.71
Sunflower silage	2	1.83	1.78	1.81	1.70

[1] As steers A–22 and B–22, trial 14, gave considerably lower values, 1.74 and 1.70 therms per pound of digestible organic matter, respecitvely, this summary with these steers omitted is given.

Armsby (*1*) gives values of 1.996 to 2.177 therms per pound of digestible organic matter in oil meals and materials high in protein. The values in table 12 agree with the lower values given by Armsby. The average value for barley 3 parts and linseed meal 1 part is close to the values directly determined for the roughages and mixed rations, but is slightly lower. The value for the wild oats seems too high, but no reason can be given for this. The value for alfalfa 5 parts and linseed meal 2 parts is 1.79 therms, which is similar to the values obtained for mixed rations and is practically identical with the directly determined value of 1.779 therms for alfalfa hay 7 parts and linseed meal 2 parts (table 11), trial 17. The indirectly computed value for the sunflower silage is 1.81 therms compared to the single directly determined value of 1.67 therms (table 11), trial 13. The directly determined value for the sunflower silage is lower than the value for the other silages, but the indirectly computed values are higher. The differences between the directly determined and the computed values are undoubtedly due, at least in part, to applying the values obtained with one set of steers on a single feed to the data obtained with other steers on mixed rations. However, the data in general confirm Armsby's observations on the uniformity of metabolizable energy in feeds per unit of digestible organic matter.

SUMMARY

In composition the silages made from sweetclover, sunflowers, and corn were generally higher in ash, crude protein, crude fiber, and ether extract, but lower in nitrogen-free extract than the crops when ensiled. The protein nitrogen ranged from 57.02 to 86.19 percent of the total nitrogen in the crops when ensiled and from 34.50 to 60.75 percent in the silages.

Compared on a dry-matter basis, the sweetclover silages and hays resembled alfalfa hay in percentage of digestibility, digestible nutrients, and metabolizable energy; but the sweetclover silages averaged a little higher in digestible crude protein and total digestible nutrients. Mixing 1 part of dry oat straw with 5.5 parts of green sweetclover reduced the digestible crude protein 56 percent and total digestible nutrients 37 percent in the silage, on dry basis, as compared to the straight sweetclover silage from the same crop (table 9).

On a dry basis (table 9), the straight sweetclover silage contained an average of 16.2 percent of digestible protein and 56.8 percent of

total digestible nutrients, compared to 5.3 percent of digestible protein and 69.7 percent of total digestible nutrients in the corn silage. The sunflower silage contained 4.8 percent of digestible protein and 47.7 percent of total digestible nutrients.

The sunflower silages were less palatable than the sweetclover silages, and the sweetclover silages were less palatable than the corn silages.

The linseed meal, dry basis, contained 32.9 percent of digestible protein and 75.8 percent of total digestible nutrients, compared to Morrison's (*8*) averages of 33.5 and 85.7 percent, respectively, for old process linseed meal.

The wild oats, dry basis, contained 9.6 percent of digestible protein and 61.6 percent of total digestible nutrients, compared to Morrison's averages of 10.3 and 78.5 percent, respectively, for common oats.

The metabolizable energy per pound of digestible organic matter in the different feeds and rations was fairly uniform. The average of 25 individual determinations on roughages was 1.737 therms per pound and 1.767 therms for 21 mixed rations, compared to 1.60 therms for roughages and 1.66 therms for mixed rations, as given by Armsby (*1*). The computed values for linseed meal and other concentrates are similar to Armsby's values.

In 25 individual trials the average metabolizable energy per pound of total digestible nutrients was 1.670 therms, and in 21 trials on mixed rations, it was 1.689 therms.

LITERATURE CITED

(1) Armsby, H. P.
1917. The nutrition of farm animals. 743 pp., illus. New York.

(2) Brody, S., Proctor, R. C., and Ashworth, U. S.
1934. Growth and development with special reference to domestic animals. XXXIV. Basal metabolism, endogenous nitrogen, creatinine and neutral sulphur excretions as functions of body weight. Mo. Agr. Expt. Sta. Research Bull. 220, 40 pp., illus.

(3) Forbes, E. B., Braman, W. W., Kriss, M., Fries, J. A., Jeffries, C. D., Swift, R. W., French, R. B., and Maucher, J. V., Jr.
1927. Net-energy values of corn silage, soy-bean hay, alfalfa hay, and oats. Jour. Agr. Research 34: 785–796.

(4) Griswold, D. J.
1933. Lamb fattening trials. N. Dak. Agr. Expt. Sta. Bull. 274, 28 pp., illus.

(5) Hopper, T. H.
1930. A rapid bulk-sample drier. Indus. and Engin. Chem., Analyt. Ed. 2: 198, illus.

(6) Kriss, M.
1931. Editorial review. A comparison of feeding standards for dairy cows, with especial reference to energy requirements. Jour. Nutrition 4: 141–161.

(7) Mitchell, H. H.
1926. The determination of the protein requirements of animals and of the protein values of farm feeds and rations. Report of the subcommittee on animal nutrition. Bull. Natl. Research Council 55, (v. 11, pt. 1), 44 pp.

(8) Morrison, F. B.
1936. Feeds and feeding.... Ed. 20, unabridged, 1050 pp., illus. Ithaca, N. Y.

(9) Titus, H. W.
1927. The nitrogen metabolism of steers on rations containing alfalfa as the sole source of the nitrogen. Jour. Agr. Research 34: 49–58, illus.

THE RELATION OF COLOR AND CAROTENE CONTENT OF ROUGHAGE IN THE DAIRY RATION TO THE COLOR, CAROTENE CONTENT, AND VITAMIN A ACTIVITY OF BUTTERFAT [1]

By R. E. HODGSON, *agent, Bureau of Dairy Industry, United States Department of Agriculture, and assistant dairy husbandman, Washington Agricultural Experiment Station;* J. C. KNOTT, *associate dairy husbandman,* and H. K. MURER, *research dairy chemist, Washington Agricultural Experiment Station;* and R. R. GRAVES, *chief, Division of Dairy Cattle Breeding, Feeding, and Management, Bureau of Dairy Industry, United States Department of Agriculture* [2]

INTRODUCTION AND REVIEW OF LITERATURE

In western Washington the majority of the pastures remain green and the grasses grow fairly rapidly throughout the summer. This insures a relatively high carotene consumption by dairy cows during the summer. In the winter, however, much of the hay fed is of inferior quality and of poor color and low carotene content, even though it is made from material originally high in color and carotene.

It is evident from research by Meigs and Converse (*15*),[3] Hart and Guilbert (*9*), and Guilbert and Hart (*8*) that dairy cattle require vitamin A or carotene in the ration for the purpose of sustaining normal health, growth, and reproduction. Kennedy and Dutcher (*13*) were among the first to report that the vitamin A value of milk is influenced by the vitamin A value of the feed, an observation which has since been confirmed by numerous investigators.

Moore (*17*) has shown that the cow converts carotene into vitamin A. The vitamin A activity of roughage in general has been shown to be related to its carotene content. Guilbert (*7*) has used the carotene analysis as a measure of vitamin A activity.

Since the roughage part of the dairy ration is the principal source of the carotene or vitamin A factor, farmers must guard against destruction of the carotene in providing roughages for their herds. Krauss (*14*), Watson (*26*), and others have shown that pasture is potent in the vitamin A factor and produces a milk of high vitamin A activity. The carotene content of roughages may vary considerably. Some of the more important factors causing variations in carotene content or in vitamin A activity are stage of maturity (*4, 12*), method of drying (*10, 20*), variety of plant (*11, 15*), and storage (*5*).

Russell and associates (*21*) and others have shown that silage may be a good source of vitamin A in the dairy ration. Peterson and coworkers (*19*) reported that the feeding of A. I. V. silage to cows produced a materially higher carotene and vitamin A content in the milk than did a check ration containing no silage.

[1] Received for publication April 19, 1938, issued October, 1938. Scientific paper No. 367, College of Agriculture and Agricultural Experiment Station, State College of Washington.

[2] The writers wish to acknowledge the cooperation in this work of V. L. Miller, chemist, Western Washing Experiment Station, and the cooperation of the Bureau of Agricultural Economics, U. S. Department of Agriculture.

[3] Italic numbers in parentheses refer to Literature Cited, p. 527.

Graves and associates (*6*) observed a relationship between the green color of hay and the color of the butterfat, while Converse, Wiseman, and Meigs (*3*) state that the color of butterfat varies definitely with the green color and the carotene content of the roughage fed.

The work herein reported was undertaken to determine the carotene content of feeds similar to those generally fed to dairy herds in this section and its relation to the color, carotene content, and vitamin A activity of the butterfat produced by cows receiving rations consisting exclusively of these feeds.

EXPERIMENTAL PROCEDURE

The roughage feeds studied in these experiments were pasture grass (grazed), field-cured hay made from perennial grass and clover mixtures, silage made from the grass and clover mixtures, and silage made from oats and peas.

When a crop was to be made into hay, it was cut after the dew had dried off and was allowed to lie in the windrow until thoroughly wilted after which it was raked and cocked. The hay remained in the cock until it was dry enough to be stored in the barn. Rain sometimes made it necessary to turn the cocks to facilitate drying. The method of making hay approximated the farm practices in this section.

The first year the forage made into silage was allowed to wilt before it was put into the silo. The second year ensiling was begun immediately after the forage was cut. Silage was made both in the ordinary upright silo and in the "stack" silo.

Thirty Holstein-Friesian cows that were being used in a general investigation of the nutritive value of home-grown all-roughage rations were being fed the roughages studied in these experiments. In the winter one group of 10 cows was maintained on the hay, a second group of 10 cows was maintained on the silage, and a third group of 10 cows was fed a ration containing both hay and silage. In the summer all three groups were maintained on pasture alone. No supplements other than salt and iodine were fed.

The crops from which the samples of roughage were taken and analyzed for their carotene and color content were grown in 1934 and 1935, and fed during 1934, 1935, and 1936. Each summer three samples of pasture grass were obtained at approximately 14-day intervals. In 1934, the first sample was taken May 24 and in 1935, on June 26. During the two winter feeding periods the hay and silage samples were also taken at approximately 14-day intervals, at the time of feeding, and represented the regular ration as it came from the haymow or from the silos. In 1934, the first samples were taken on December 14 and in 1935, on December 27. Each year the cows were receiving hay or silage rations at least 90 days before the samples were taken.

Carotene and color analyses were made on samples of the butterfat produced by cows receiving the various roughages. The butterfat samples were obtained from the evening's milk on the day the feed samples were taken and from the following morning's milk. The milk from each group was from cows in different stages of lactation. The milk was separated while still warm and the cream from the evening's and morning's milk was combined. The sweet cream was churned in a small churn at a temperature of 10° to 15° C. The

freshly churned butter was melted at 60°, centrifuged, and the pure fat decanted into glass containers. These samples were analyzed for carotene and color and fed to rats to determine their vitamin A value.

All samples of feed and butterfat were frozen as soon after they were taken as possible. The samples were packed in solid carbon dioxide and held in it, or at −20° C. or below, until prepared for analysis.

Carotene determinations were made on duplicate samples of the feed by a modification of the methods of Schertz (*22*) and Miller (*16*). The first extraction of the pigments was made by grinding the samples in a ball mill from which the air was excluded with carbon dioxide. The samples were covered with acetone during the grinding and the apparatus was kept at 0° C. throughout the process. After the samples were ground the extractions were completed with acetone and ether. The pigment concentration, after fractionation, was determined by means of an Exton-photoelectric scopometer previously calibrated with pure beta-carotene. The pigments were extracted from the butterfat with ethyl ether after saponification. The pigments were then fractionated and determined in the same manner as for the feeds. The color determinations both of the feed and the butterfat were made by the Bureau of Agricultural Economics, United States Department of Agriculture according to the method described by Nickerson (*18*).

Three experiments were run to determine the vitamin A value of the butterfat. Experiment 1 was conducted in the winter of 1934 on samples of butterfat produced by cows on the hay alone, on the silage alone, and on the hay and silage. Experiment 2 was conducted in the summer of 1935 on samples of butterfat produced by cows on pasture. Experiment 3 was conducted in the winter of 1935, and duplicated experiment 1.

Determinations of the vitamin A value of the butterfat samples were made by a modified Sherman (*24*) technique, as follows: Young albino rats from mothers receiving a diet with a standardized vitamin A content were used in each experiment. After 21 days of age they were maintained on a vitamin A-free diet consisting of heated casein, 18 percent; dry yeast, 10 percent; Osborne and Mendel salt mixture, 4 percent; sodium chloride, 1 percent; and heated cornstarch, 67 percent. The casein was heated for 108 hours at 105° C. Vitamin D was supplied by the addition of 0.02 percent of 250 D viosterol. When the animals were depleted of their body stores of vitamin A, as indicated by a stationary or declining weight for 4 consecutive days, they were placed in individual cages and the basal ration was supplemented with a butterfat sample, fed twice a week over a period of 5 weeks. The butterfat was fed on watch crystals. The rats were distributed among the various groups according to the suggestions of Sherman and Burtis (*23*). With each experiment a representative group of rats was maintained on the basal diet as a negative control.

In the second and third experiments the technique was modified to include in each experiment two representative groups of rats which received supplements of the U. S. P. x (1934) reference cod-liver oil (*25*) containing 3,000 units of vitamin A per gram, mixed in coconut oil. The reference oil was given twice a week directly into the mouth by means of a syringe. In each experiment, one lot was fed at the

rate of 1.43 and the other at the rate of 2.00 units of vitamin A per day. The reference mixtures were made up every 10 days and were held in a refrigerator between feeding periods.

EXPERIMENTAL RESULTS

Six samples of pasture grass, and 12 samples each of the hay ration, of the silage ration, and of the hay and silage ration, were available for study of the realtion between the carotene content of the feeds and the color and carotene of the butterfat.

Pasture grass sample Nos. 1, 2, and 3 were obtained during 1934 and Nos. 4, 5, and 6 during 1935. Hay or silage samples 1 to 5, inclusive, represent the 1934 crop and samples 6 to 12, inclusive, represent the 1935 crop. Sample Nos. 4 and 5 and 11 and 12 were silage made from oats and peas, the remainder being silage made from the same type of material as was the hay.

Color measurements for roughage were made only for the hay. The relationship of these various factors in the feed and butterfat is shown in table 1, the feed samples in each ration being arranged according to their carotene content, in descending order from the lowest to the highest.

There appears to be a general relationship between the green color of the hay and its carotene content, that is, the higher the color the higher the carotene content, but the relationship between these factors was not consistent.

In none of the rations was there a consistent relationship between the carotene content of the feed sample and that of the butterfat, nor between the carotene content of the butterfat and its color.

TABLE 1.—*Relation of the color and carotene content of roughage to the color and carotene content of butterfat produced by cows on the different rations*

HOME-GROWN HAY

Sample No.[1]	Roughage		Butterfat	
	Color [2]	Carotene content per gram (dry-matter basis)	Carotene content per gram (dry-matter basis)	Color index chroma reading
	Percent	*Micrograms*	*Micrograms*	
4	34	8	2.6	4.7
5	39	9	2.8	3.9
2	32	12	2.8	5.0
1	41	12	3.0	4.3
3	40	13	3.0	4.2
7	52	16	4.5	4.9
9	60	17	4.3	4.4
10	59	17	4.2	5.5
11	53	18	3.6	5.0
6	52	18	4.5	4.6
8	46	19	3.7	4.6
12	55	19	3.6	4.6
Mean		14.8	3.55	4.64

[1] Sample numbers represent the order in which the feeds were fed. In the hay, hay and silage, and silage ration samples 1 to 5, inclusive, represent the 1934 crop and samples 6 to 12, inclusive, represent the 1935 crop. Pasture samples 1, 2, and 3 were taken in 1934, and 4, 5, and 6 in 1935.

[2] Indicates percentage of the natural green color.

TABLE 1.—*Relation of the color and carotene content of roughage to the color and carotene content of butterfat produced by cows on the different rations*—Continued

HAY AND SILAGE

Sample No.	Roughage		Butterfat	
	Color	Carotene content per gram (dry-matter basis)	Carotene content per gram (dry-matter basis)	Color index chroma reading
	Percent	*Micrograms*	*Micrograms*	
10		31	7.3	6.1
11		41	5.6	5.9
9		43	7.2	5.2
8		45	7.5	5.9
12		49	5.9	5.8
7		50	8.0	6.1
4		54	6.0	5.3
1		56	6.0	5.2
2		58	6.0	5.8
5		75	5.9	5.3
6		87	7.9	6.0
3		95	5.2	5.3
Mean		57.0	6.54	5.66

SILAGE

1		107	6.4	5.7
4		109	6.5	5.6
2		134	6.4	5.8
5		156	6.0	6.0
10		157	7.5	6.2
3		199	5.1	5.0
11		205	6.8	5.5
9		233	6.7	5.7
8		251	6.1	5.8
12		260	7.2	6.0
7		268	8.2	5.8
6		288	8.3	6.3
Mean		197.3	6.77	5.78

PASTURE

2		220	7.4	6.0
3		256	7.6	5.4
6		258	9.1	6.0
1		266	6.5	5.9
5		273	8.1	5.8
4		286	8.7	5.7
Mean		260.0	7.9	5.8

Comparison of the average values, however, shows that as the carotene content of the ration increased there was an increase in the carotene and color of the butterfat. The increase in the butterfat was not in direct proportion to the increase in the ration, however. The average carotene content of the pasture grass was 17.6 times as high as that of the hay ration; the silage was 13.3 times as high; and the hay and silage was 3.8 times as high; whereas the carotene content of the butterfat produced on pasture, on silage, and on hay and silage, was only 2.2, 1.9, and 1.8 times as high, respectively, as that produced on hay.

The relation between the carotene content and color of the 42 samples of butterfat is shown graphically in figure 1. The spread in color index of the butterfat, from the high to the low, is not so great as the spread in carotene content.

A summary of the observations during the 2-year experimental feeding period shows the relative effectiveness of the different kinds of roughage in producing butterfat with a high carotene content, when fed as the sole ration. Considering the effectiveness of the home-grown field-cured hay as 1.00, the comparative results were as follows:

Roughage, and its treatment:	*Relative carotene value of the butterfat*
Hay, home-grown, field-cured	1.00
Hay, plus oat and pea silage (silo)	1.75
Grass silage (silo)	1.88
Hay, plus grass silage (silo)	1.90
Oat and pea silage (silo)	1.99
Hay, plus grass silage (stack)	2.00
Grass silage (stack)	2.07
Pasture grass (grazed)	2.22

Although in many instances the hay and the grass silage were made from the same type of crop material, harvested at approximately the same time, the hay contained much less carotene than the silage. This would indicate that the haymaking and hay-storing practices were very destructive to carotene. The high carotene content of the silages studied would appear to indicate that ensiling a crop is an effective way to produce feeds of high carotene content for winter feeding.

From the data at hand it is possible to calculate the percentage of ingested carotene that was transmitted to the butterfat unchanged. Table 2 shows the average daily carotene intake by the cows on the hay ration, the silage ration, and the hay and silage ration, also the percentage of the carotene intake that was recovered as such in the butterfat.

On an average, the higher the daily carotene intake the lower the percentage of the intake that is transmitted unchanged to the butterfat. Furthermore, at any level of carotene intake the percentage transmitted is very small. Even in the hay ration, in which the average daily consumption of carotene was as low as 147 mg per day (see 1934 trials, table 2), the percentage transmitted was less than 1 percent. The average carotene content of both the hay and silage was much less in 1934 than in 1935. The percentages of ingested carotene transmitted unchanged to the butterfat, indicated by this study, while somewhat lower than the percentages reported by Baumann and associates (*1*), tend to confirm their results.

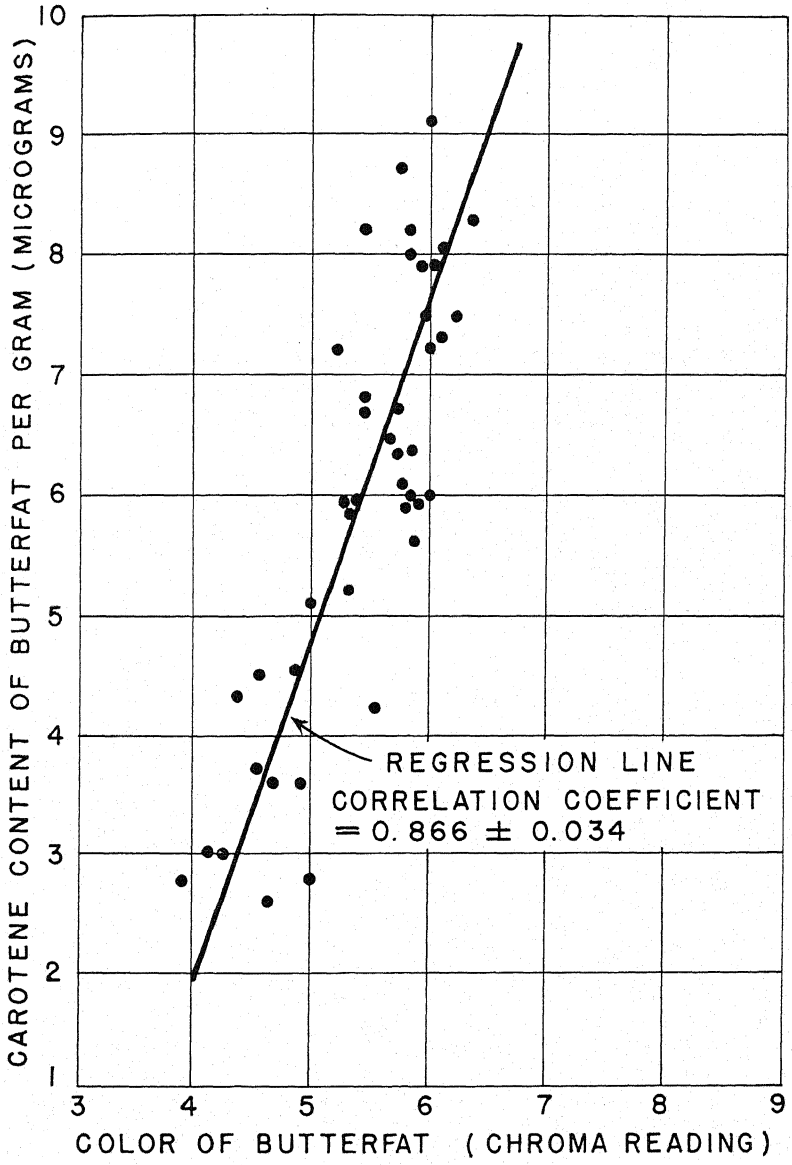


FIGURE 1.—Relation of carotene content of butterfat to its color.

TABLE 2.—*Average daily consumption of carotene per cow on rations of hay alone, hay and silage, and silage alone (dry-matter basis); also the percentage of the carotene intake that was recovered as such in the butterfat*

HAY RATION

Crop year, and sample No.	Cows	Hay			Silage			Total carotene intake	Butterfat			Percentage of the carotene intake recovered in the butterfat
		Quantity fed	Carotene content per gram	Carotene consumed	Quantity fed	Carotene content per gram	Carotene consumed		Yield	Carotene content per gram	Total carotene	
1934:	*Number*	*Kilograms*	*Micrograms*	*Milligrams*	*Kilograms*	*Micrograms*	*Milligrams*	*Milligrams*	*Kilograms*	*Micrograms*	*Milligrams*	*Percent*
1	5	13.11	12.4	162.5				162.5	0.368	3.1	1.14	0.70
2	5	13.52	12.2	164.9				164.9	.430	2.8	1.20	.73
3	5	13.65	13.2	180.1				180.1	.410	3.0	1.23	.67
4	5	13.38	7.6	101.7				101.7	.381	2.6	.99	.97
5	5	13.47	9.4	126.6				126.6	.313	2.8	.88	.69
Average	5	13.43	11.0	147.2				147.2	.380	2.9	1.09	1.74
1935:												
6	8	12.16	18.0	218.8				218.8	.364	4.5	1.64	.75
7	8	12.16	16.0	194.5				194.5	.364	4.5	1.64	.84
8	9	11.79	19.0	224.0				224.0	.408	3.7	1.51	.67
9	9	11.93	17.0	202.8				202.8	.381	4.3	1.64	.81
10	10	11.88	17.0	202.0				202.0	.335	4.2	1.41	.70
11	9	11.16	18.0	200.9				200.9	.313	3.6	1.13	.56
12	8	12.79	19.0	243.1				243.1	.352	3.6	1.27	.52
Average	8.7	11.98	17.7	212.3				212.3	.360	4.1	1.46	1.69
Grand average	7.2	12.58	14.9	185.2				185.2	.368	3.6	1.31	1.71

HAY AND SILAGE RATION

1934:												
1	7	6.35	12.4	78.7	5.31	107	567.1	645.8	0.386	6.0	2.32	0.36
2	7	6.67	12.2	81.4	4.04	134	541.4	617.4	.344	6.0	2.06	.34
3	7	6.85	13.2	90.4	5.22	199	1,038.8	1,125.0	.310	5.2	1.61	.14
4	7	6.49	7.6	49.3	5.53	109	603.9	648.8	.297	6.0	1.78	.27
5	6	6.40	9.4	60.1	4.76	156	742.6	808.9	.274	5.9	1.62	.20
Average	6.8	6.55	11.0	72.0	4.97	141	698.8	770.8	.322	5.8	1.88	1.24

1935:												
6	9	7.57	18.0	136.3	4.63	288	1,333.4	1,461.1	.338	7.9	2.67	.18
7	9	7.57	16.0	121.1	4.63	268	1,240.8	1,353.9	.338	8.0	2.70	.20
8	10	7.53	19.0	143.1	3.99	251	1,001.5	1,147.1	.369	7.5	2.77	.24
9	10	7.57	17.0	128.7	4.26	233	992.6	1,130.6	350	7.2	2.52	.22
10	9	7.98	17.0	135.7	4.54	157	712.8	842.2	.348	7.3	2.54	.30
11	8	6.89	18.0	124.0	4.40	205	902.0	1,026.0	.327	5.6	1.83	.18
12	8	7.12	19.0	135.3	4.17	260	1,084.2	1,227.3	.323	5.9	1.91	.16
Average	9	7.46	17.7	132.0	4.37	237	1,038.2	1,170.2	.342	7.0	2.42	[1].21
Grand average	8.1	7.08	14.9	107.0	4.62	197	896.8	1,003.8	.334	6.5	2.19	[1].22

SILAGE RATION

1934:												
1	6				6.76	107	723.3	723.3	0.351	6.4	2.25	0.22
2	6				7.94	134	1,063.9	1,063.9	.421	6.4	2.69	.25
3	6				10.39	199	2,067.8	2,067.8	.328	5.1	1.67	.08
4	6				11.48	109	1,251.3	1,251.3	.335	6.5	2.18	.17
5	6				10.57	156	1,648.9	1,648.9	.244	6.0	1.46	.09
Average	6				9.43	141	1,351.0	1,351.0	.336	6.1	2.05	[1].15
1935:												
6	7				10.88	288	3,133.4	3,133.4	.317	8.3	2.63	.08
7	7				10.88	268	2,915.8	2,915.8	.317	8.2	2.60	.09
8	8				9.48	251	2,379.5	2,379.5	.391	6.1	2.39	.10
9	9				8.98	233	2,092.3	2,092.3	.391	6.7	2.49	.12
10	10				7.03	157	1,103.7	1,103.7	.375	7.5	2.81	.26
11	10				7.39	205	1,514.9	1,514.9	.259	6.8	1.76	.12
12	10				6.53	260	1,697.8	1,697.8	.229	7.2	1.65	.10
Average	8.7				8.74	237	2,119.6	2,119.6	.326	7.3	2.33	[1].11
Grand average	7.6				9.02	197	1,799.4	1,799.4	.330	6.8	2.22	[1].12

[1] Percentage based on weighted average.

The relation between average daily carotene consumption and the percentage of the ingested carotene transmitted unchanged to butterfat, based on 36 observations, is shown graphically in figure 2.

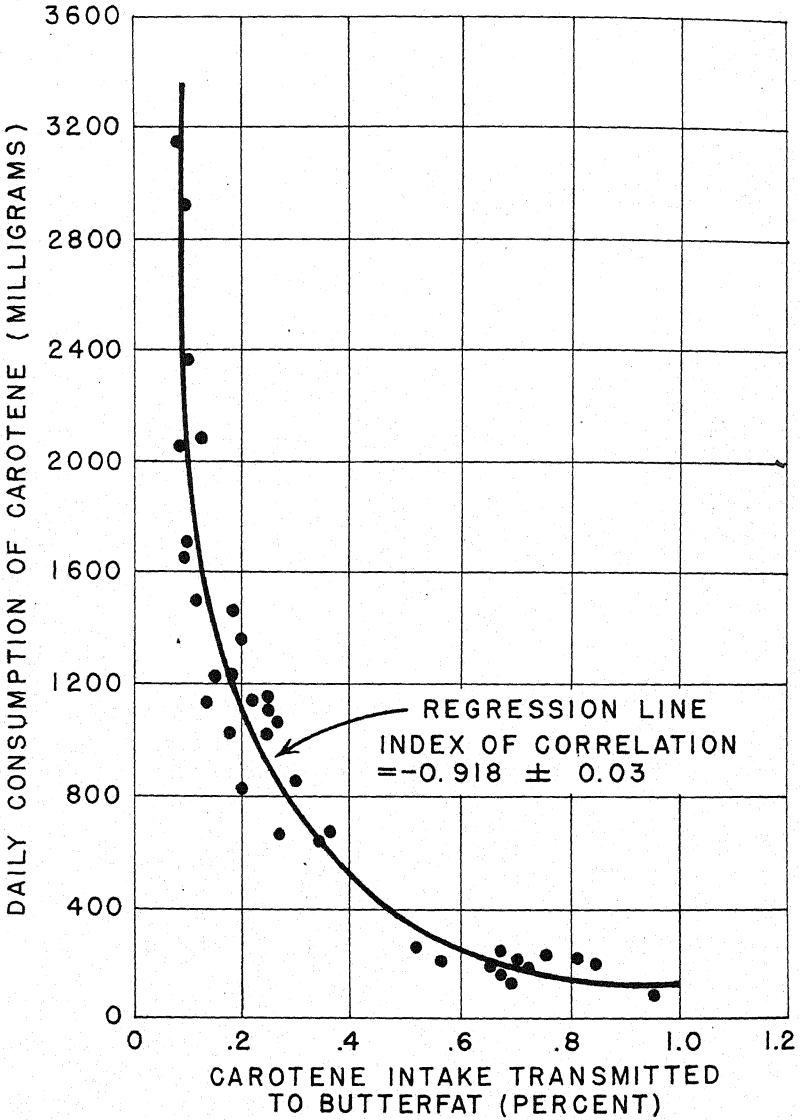


FIGURE 2.—Relation between daily carotene intake and the percentage of the intake transmitted to butterfat in the form of carotene, based on 36 observations.

Table 3 shows the growth made by rats fed different amounts of the different samples of butterfat, also the apparent vitamin A activity of each sample. The vitamin A activity was calculated on the basis of the average growth made by reference groups of rats in experiments 2 and 3 that were fed a known number of vitamin A units in U. S. P.x

reference oil. In each of the three experiments representative groups of animals were maintained on the basal diet as negative controls. In the first experiment seven rats survived an average of 11 days and lost an average of 9 g. In the second experiment 10 rats survived an average of 15 days and lost 17 g. In the third experiment seven rats survived an average of 15 days and lost 19 g.

TABLE 3.—*Vitamin A content of butterfat produced by cows on different roughage rations, as determined on the basis of U. S. P.x (1934) reference cod-liver oil and also by the Sherman unit growth method*

Ration fed to cows producing the butterfat	Experimental rats: Lot No.	Experimental rats: Per lot	Amount of butterfat fed daily	Average gain per rat in 5-week period	Factor for gain per unit of vitamin A	Vitamin A fed in butterfat or cod-liver oil	Vitamin A per gram of butterfat by—: U. S. P.x method	Vitamin A per gram of butterfat by—: Sherman method	Decrease by U. S. P.x method:	
		Number	*Milligrams*	*Grams*	*Grams*	*Units*	*Units*	*Units*	*Units*	*Per cent*
EXPERIMENT 1 (1934)										
Hay	1	10	40	36.6	[1] 0.575	63.6	45.5	50.8	5.3	10.4
Hay	2	9	80	64.0	[1] .575	111.3	39.8	44.5	4.7	10.6
Hay	3	10	160	83.1	[1] .575	144.5	25.8	28.9	3.1	10.7
Silage	4	10	40	61.0	[1] .575	106.1	75.8	84.8	9.0	10.6
Silage	5	9	80	82.7	[1] .575	143.8	51.4	57.4	6.0	10.5
Silage	6	11	160	101.2	[1] .575	176.0	31.4	35.1	3.7	10.5
Hay and silage	7	9	40	60.3	[1] .575	104.9	74.9	83.8	8.9	10.6
Hay and silage	8	10	80	81.4	[1] .575	141.6	50.6	56.5	5.9	10.4
Hay and silage	9	10	160	89.0	[1] .575	154.8	27.6	30.9	3.3	10.7
EXPERIMENT 2 (1935)										
Pasture	1	9	10	7.1	[2] 0.595	11.9	34.0	39.4	5.4	13.7
Pasture	2	9	20	43.8	[2] .595	73.6	105.1	122.0	16.9	13.9
Pasture	3	10	40	63.8	[2] .595	107.2	76.6	88.5	11.9	13.5
	[3] 4	10	[4]	30.7	.600	50.5	----------	----------	------	------
	[3] 5	10	[5]	41.0	.590	70.0	----------	----------	------	------
EXPERIMENT 3 (1935)										
Hay	1	8	21.5	9.1	[6] 0.555	16.4	21.8	23.5	1.7	7.2
Hay	2	8	43.0	46.5	[6] .555	83.8	55.9	60.1	4.2	7.0
Silage	3	8	21.5	49.2	[6] .555	88.6	117.8	127.1	9.3	7.3
Silage	4	8	43.0	76.8	[6] .555	138.4	92.3	99.2	6.9	7.0
Hay and silage	5	7	21.5	40.4	[6] .555	72.8	96.8	104.2	7.4	7.1
Hay and silage	6	8	43.0	90.8	[6] .555	163.6	109.1	117.2	8.1	6.9
	[3] 7	7	[4]	25.3	.510	50.5	----------	----------	------	------
	[3] 8	7	[5]	41.7	.600	70.0	----------	----------	------	------

[1] Based on the average of the factors for gain per unit of vitamin A by lots 4 and 5 in experiment 2 and lots 7 and 8 in experiment 3.
[2] Based on the average of the factors for gain per unit of vitamin A by lots 4 and 5 in experiment 2.
[3] Received known amounts of vitamin A in the form of U. S. P.x reference cod-liver oil.
[4] Received 1.43 units of vitamin A daily in cod-liver oil.
[5] Received 2.00 units of vitamin A daily in cod-liver oil.
[6] Based on the average of the factors for gain per unit of vitamin A by lots 7 and 8 in experiment 3.

As shown in table 3, the vitamin A values for the experiments were calculated by both the Sherman weight-gain method and the U. S. P.x reference oil method. The vitamin A values for the butterfat were approximately 14 percent lower in experiment 2 and 7 percent lower in experiment 3 when calculated by the U. S. P.x method.

There was a high degree of uniformity in the growth response by the reference groups in experiments 2 and 3. In experiment 2, lots 4 and 5 gained 0.60 and 0.59 g, respectively, in the 5-week period, or an average of 0.595 g per unit of vitamin A fed in cod-liver oil. In experiment 3,

lots 7 and 8 gained 0.51 and 0.60 g, respectively, or an average of 0.555 g per unit of vitamin A fed. The average for all four reference lots was 0.575 g. This factor was used as a basis for calculating the U. S. P.x vitamin A values in experiment 1.

As will be noted by a study of table 3, the different groups of rats did not all produce growth responses in proportion to the amount of butterfat fed. Therefore, the comparative vitamin A activity of the butterfats produced on the different roughage rations has been interpreted by selecting from table 3 the groups of rats that produced a growth response most closely approximating that of the reference groups. Table 4 shows the average vitamin A activity of butterfat produced on hay, on silage, on hay and silage, and on pasture, as indicated by the selected groups of rats.

TABLE 4.—*Average vitamin A activity of butterfat produced on hay, on hay and silage, on silage, and on pasture, as indicated by selected groups of rats*

Ration fed to cows producing the butterfat	Experiment No.	Lot No.	Rats per lot	Amount of butterfat fed daily	Average gain per rat in 5-week period	Gain per unit of vitamin A by reference groups	Vitamin A per gram of butterfat
			Number	*Milligrams*	*Grams*	*Grams*	*Units*
Hay	1	1	10	40	36.6	0.575	45
	3	2	8	43	46.5	.555	56
Average							50
Hay and silage	1	7	9	40	60.3	.575	75
	3	6	7	21.5	40.4	.555	97
Average							86
Silage	1	4	9	40	61.0	.575	76
	3	3	8	21.5	49.2	.555	118
Average							97
Pasture	2	2	9	20	43.8	.595	105

It is apparent that pasture grass produced butterfat more than twice as high in vitamin A activity as did the hay ration; while the silage produced butterfat 1.9 times as high in vitamin A, and the hay and silage 1.7 times as high as the hay.

The total daily production of vitamin A per cow in the butterfat produced on the various rations, as expressed in U. S. P. x (revised 1934) units, was approximately 18,000 for the cows on the hay ration, 28,000 for the cows on the hay and silage ration, and 32,000 for the cows on the all silage ration.

Table 5 has been prepared to show the relationship of the carotene content of butterfat to its total vitamin A activity. For this purpose 0.6 microgram of carotene has been taken as equivalent to one U. S. P. x (revised 1934) unit of vitamin A. While this equivalence has been questioned in the case of butterfat (*2*) its use gives relative figures which show a rather constant ratio of carotene content to vitamin A activity in the butterfat. It appears that regardless of the amount of carotene in the ration these cows maintained a relatively constant ratio of carotene to total vitamin A activity in their butterfat.

TABLE 5.—*Average carotene content of butterfat produced on four different roughage rations, and the percentage of total vitamin activity in the butterfat attributable to the carotene in the butterfat*

Ration fed to cows producing the butterfat	Experiment No.	Average carotene content of butterfat per gram	Vitamin A attributed to carotene in butterfat		Total vitamin A activity of butterfat
		Micrograms	*Units* [1]	*Percent*	*Units*
Hay	1	2.83	4.7	10.35	45
	3	4.05	6.7	11.96	56
Average		3.44	5.7	11.16	50
Hay and silage	1	5.71	9.5	12.68	75
	3	7.33	12.2		97
Average		6.52	10.8	12.68	86
Silage	1	5.94	9.9	13.06	76
	3	6.77	11.3	9.58	118
Average		6.36	10.6	11.32	97
Pasture	2	8.63	14.4	13.71	105

[1] 0.6 microgram of carotene considered equivalent to 1 U. S. P. x (revised 1934) unit of vitamin A.

The vitamin A activity of the butterfats did not follow proportionately the increase in the carotene content of the several rations. The same is true of the vitamin A activity with respect to the color of the butterfat. The ratio of the carotene content of the butterfat and its vitamin A activity remained practically constant for each of the four rations. While a high carotene content of the roughage and a high color content of the butterfat indicated a high vitamin A value, they were much less indicative of the vitamin A value than the carotene content of the butterfat. The carotene content of the feed may indicate in general the carotene content, or vitamin A activity, of the butterfat but it is not an accurate measure.

During the course of this experiment one of the three groups of 10 cows was maintained throughout two winters of 181 and 182 days duration, respectively, on an all-hay ration, with no evidence of vitamin A deficiency on this feeding schedule. Parturition in all cases was normal and the calves were strong, healthy, normal individuals. There is a probability of some carry-over of vitamin A from the pasture season, however. The minimum requirements of carotene for cattle, observed by Guilbert (*8*), are well below the amounts received by the cows on the hay ration.

The real advantage, therefore, in providing high-carotene feeds, above a minimum requirement which is not yet accurately known, is that it improves the vitamin A value of the butterfat in the milk. These studies demonstrate the comparative vitamin A value of the butterfat produced on the various kinds of roughage. The roughage part of a good dairy ration including both hay and silage in the winter and pasture in the summer as fed in this experiment will effectively provide sufficient of the vitamin A factor for maintenance, reproduction, and the production of butterfat of high vitamin A activity.

SUMMARY AND CONCLUSIONS

Twelve samples of the home-grown field-cured hay fed in this experiment were analyzed for their green color and their carotene content. In general, the samples containing the highest percentages of green color also were highest in carotene content. This relationship did not hold true for each of the 12 samples, however.

The average carotene content of the hay ration, the hay and silage ration, the grass-silage ration, and the pasture ration was 14.8 micrograms per gram, 57.0 micrograms per gram, 197.3 micrograms per gram, and 260 micrograms per gram, respectively. When these rations were fed as the sole roughage to Holstein-Friesian cows, they produced butterfat with an average carotene content of 3.6 micrograms per gram for hay; 6.5 micrograms per gram for hay and silage; 6.8 micrograms per gram for silage; and 7.9 micrograms per gram for pasture. The increase in the carotene content of the butterfat was not in the same proportion as the increase in the carotene content of the feeds.

There was a good relationship between the color and carotene content of butterfat. For 42 observations the correlation coefficient was 0.866 ± 0.034.

The home-grown field-cured hay fed in the winter, which had an average carotene content of 14.8 micrograms per gram in addition to whatever carry-over there might have been from pasture the preceding summer, apparently furnished enough of the vitamin A factor for normal body activities, reproduction, and the production of butterfat of approximately 50 units per gram. The average daily carotene intake was 185 mg.

A ration of home-grown hay and grass silage with an average carotene content of 57 micrograms per gram produced a butterfat with a vitamin A value of approximately 86 units per gram. The average daily carotene intake was 897 mg.

A ration of grass silage with an average carotene content of 197 micrograms per gram produced a butterfat with a vitamin A value of approximately 97 units per gram. The average daily carotene intake was 1,799 mg.

Pasture with an average carotene content of 260 micrograms per gram produced a butterfat of approximately 105 units per gram.

The average percentage of the carotene ingested that was secreted as carotene in the butterfat was 0.71 percent for the cows receiving hay, 0.22 percent for the cows receiving hay and silage, and 0.12 percent for the cows receiving silage.

The carotene in butterfat accounted for only a small percentage of the total vitamin A activity. By assuming that 0.6 microgram of carotene is equivalent to one U. S. P. x (1934 revision) unit of vitamin A the percentage of the vitamin A attributable to carotene in the hay ration was 11.2 percent; hay and silage ration 12.7 percent; silage ration 11.3 percent; and pasture 13.7 percent.

LITERATURE CITED

(1) BAUMANN, C. A., STEENBOCK, H., BEESON, W. M., and RUPEL, I. W.
1934. FAT-SOLUBLE VITAMINS. XXXIX. THE INFLUENCE OF BREED AND DIET OF COWS ON THE CAROTENE AND VITAMIN A CONTENT OF BUTTER. Jour. Biol. Chem. 105: 167–176.

(2) BOOTH, ROLAND GORDON, KON, STANISLAW KAZIMIERZ, and GILLAM, ALBERT EDWARD.
1934. THE RELATIVE BIOLOGICAL EFFICIENCIES OF THE VITAMIN A AND CAROTENE OF BUTTER. Biochem. Jour. 28: [2169]–2174.

(3) CONVERSE, H. T., WISEMAN, H. G., and MEIGS, E. B.
1935. THE RELATION BETWEEN THE COLOR AND VITAMIN A OF BUTTER AND THE FEED OF THE COW. Amer. Soc. Anim. Prod. Proc. (1934) 27: 50–54.

(4) DOUGLASS, EARL, TOBISKA, J. W., and VAIL, C. E.
1933. STUDIES ON CHANGES IN VITAMIN CONTENT OF ALFALFA HAY. Colo. Expt. Sta. Tech. Bull. 4, 68 pp., illus.

(5) FRAPS, G. S., and TREICHLER, RAY.
1933. EFFECT OF STORAGE ON VITAMIN A IN DRIED FOODS. Indus. and Engin. Chem. 25: 465–466.

(6) GRAVES, R. R.
1933. FEEDING AND MANAGEMENT INVESTIGATIONS AT BELTSVILLE. U. S. Bur. Dairy Indus. Rept. Chief 1933: 9–10.

(7) GUILBERT, H. R.
1934. DETERMINATION OF CAROTENE AS A MEANS OF ESTIMATING THE VITAMIN A VALUE OF FORAGE. Indus. and Engin. Chem., Analyt. Ed. 6: 452–454.

(8) ——— and HART, G. H.
1935. MINIMUM VITAMIN A REQUIREMENTS WITH PARTICULAR REFERENCE TO CATTLE. Jour. Nutrition 10: 409–427.

(9) HART, G. H., and GUILBERT, H. R.
1933. VITAMIN-A DEFICIENCY AS RELATED TO REPRODUCTION IN RANGE CATTLE. Calif. Agr. Expt. Sta. Bull. 560, 30 pp., illus.

(10) HATHAWAY, I. L., DAVIS, H. P., and GRAVES, R. R.
1932. THE VITAMIN A AND THE VITAMIN E CONTENT OF FIELD-CURED AND ARTIFICIALLY CURED ALFALFA HAY. Nebr. Agr. Expt. Sta. Research Bull. 62, 15 pp.

(11) HILTON, J. H., HAUGE, S. M., and WILBUR, J. W.
1933. MAINTAINING THE VITAMIN A VALUE OF BUTTER THROUGH WINTER FEEDING CONDITIONS. Jour. Dairy Sci. 16: 355–361, illus.

(12) ——— HAUGE, S. M., and WILBUR, J. W.
1935. THE VITAMIN A ACTIVITY OF BUTTER PRODUCED BY COWS FED ALFALFA HAY AND SOYBEAN HAY CUT IN DIFFERENT STAGES OF MATURITY. Jour. Dairy Sci. 18: 795–800.

(13) KENNEDY, CORNELIA, and DUTCHER, R. A.
1922. VITAMIN STUDIES. IX. THE INFLUENCE OF THE DIET OF THE COW UPON THE QUANTITY OF VITAMINES A AND B IN THE MILK. Jour. Biol. Chem. 50: 339–359, illus.

(14) KRAUSS, W. E.
1931. EFFECT OF PASTURE ON VITAMIN-A CONTENT OF MILK. Ohio Agr. Expt. Sta Bull. 470 (Ann. Rept. 49): 149–150, illus.

(15) MEIGS, EDWARD B., and CONVERSE, H. T.
1934. VITAMIN A IN THE NUTRITION OF DAIRY CATTLE. Amer. Soc. Anim. Prod. (1933) 26: 58–61.

(16) MILLER, ELMER S.
1935. A RAPID AND ACCURATE QUANTITATIVE METHOD FOR THE DETERMINATION OF COMMON CAROTENOIDS; ANALYSIS OF BETA-CAROTENE AND LEAF XANTHOPHYLL IN THIRTEEN PLANT TISSUES. Jour. Amer. Chem. Soc. 57: 347–349.

(17) MOORE, THOMAS.
1932. VITAMIN A AND CAROTENE. IX. NOTES ON THE CONVERSION OF CAROTENE TO VITAMIN A IN THE COW. Biochem. Jour. 26: 1–9, illus.

(18) NICKERSON, DOROTHY.
1929. A METHOD FOR DETERMINING THE COLOR OF AGRICULTURAL PRODUCTS. U. S. Dept. Agr. Tech. Bull. 154, 32 pp., illus.

(19) PETERSON, W. H., BOHSTEDT, G., BIRD, H. R., and BEESON, W. M.
1935. THE PREPARATION AND NUTRITIVE VALUE OF A. I. V. SILAGE FOR DAIRY COWS. Jour. Dairy Sci. 18: 63–78, illus.

(20) RUSSELL, WALTER C., TAYLOR, M. W., and CHICHESTER, D. F.
1934. THE EFFECT OF THE CURING PROCESS UPON THE CAROTENE AND VITAMIN A CONTENT OF ALFALFA. N. J. Agr. Expt. Sta. Bull. 560, 8 pp., illus.

(21) ——— TAYLOR, M. W., CHICHESTER, D. R., and WILSON, L. T.
1935. THE RELATION BETWEEN THE VITAMIN A CONTENT OF THE DAIRY RATION AND OF MILK. N. J. Agr. Expt. Sta. Bull. 592, 11 pp.

(22) SCHERTZ, F. M.
1923. THE QUANTITATIVE DETERMINATION OF CAROTIN BY MEANS OF THE SPECTROPHOTOMETER AND THE COLORIMETER. Jour. Agr. Research 26: 383–400, illus.

(23) SHERMAN, H. C., and BURTIS, M. P.
1928. FACTORS AFFECTING THE ACCURACY OF THE QUANTITATIVE DETERMINATION OF VITAMIN A. Jour. Biol. Chem. 78: 671–680.

(24) ——— and MUNSELL, H. E.
1925. THE QUANTITATIVE DETERMINATION OF VITAMIN A. Jour. Amer. Chem. Soc. 47: 1639–1646, illus.

(25) UNITED STATES PHARMACOPOEIAL CONVENTION, COMMITTEE OF REVISION OF THE UNITED STATES PHARMACOPOEIA.
1934. THE PHARMACOPOEIA OF THE UNITED STATES OF AMERICA. 10TH DECENNIAL REVISION (U. S. P. X). U.S.P.x Interim Revision Announcement No. 2.

(26) WATSON, S. J.
1932. VITAMIN A AND CAROTENE CONTENT OF GRASS AND OF BUTTER MADE FROM THE WINTER-FED COWS FED ON ARTIFICIALLY DRIED GRASS. Jour. Soc. Chem. Indus. (Chem. and Indus.) 51: 536.

THE EFFECT OF ENVIRONMENTAL TEMPERATURE ON MORTALITY, RATE OF GROWTH, AND UTILIZATION OF FOOD ENERGY IN WHITE LEGHORN CHICKS[1]

By C. F. WINCHESTER, formerly *research assistant* and M. KLEIBER, *associate animal husbandman, California Agricultural Experiment Station*[2]

INTRODUCTION

The effect of environmental temperature on growth rate, food consumption, and the conversion of food to body substance in chicks has been investigated by Kleiber and Dougherty (*11*)[3] who studied the effects of air temperatures of 21°, 27°, 32°, 38°, and 40° C. on chicks during the period from 6 to 15 days of age. The daily rate of growth was found to increase as the environmental temperature decreased. Maximum gain in weight was 4.88 g per day at 21°, while minimum gain was 2.74 g per day at 40°. Availability of food, defined as $\frac{\text{food}-\text{excreta}}{\text{food}}$, was optimum at 38°.

Energy stored as gain in body substance (net energy in production) reached a maximum at 32° C. The energy content per unit of weight gained was at a minimum at 21° (1.41 Cal.[4] per gram) and at a maximum (2.95 Cal. per gram) at 38°. Total efficiency (the total net energy produced per unit of food energy consumed) exhibited a maximum of 34 percent at 32° and a minimum at 21°, at which temperature it was 16 percent.

Barott and his coworkers (*2*) studied the effects of environmental temperature on chicks younger than those used by Kleiber and Dougherty. Heat production and gaseous exchange of chicks from 10 to 100 hours of age, at temperatures from 68° to 104° F., were determined with a respiration calorimeter. A "critical temperature" was found at 96°, and a 15-percent increase in metabolism resulted from an increase or decrease of 7° from this temperature. Metabolism increased as environmental temperature decreased from 96° to 70°. The energy output at 70° was twice that at 96°. Deutectomized as well as normal chicks were studied.

Lewis (*13*), using groups of 50 White Leghorn chicks, found that at an average temperature of 29° C. (32° on the first day decreased gradually to 23° on the twenty-eighth day) there was a mortality of 24 percent, while there was a mortality of 10 percent at an average temperature of 34°. Lewis *(13, p. 102)* stated: "This increased death rate was due in nearly every instance to crowding at night in an effort to keep warm."

[1] Received for publication March 2, 1938; issued October, 1938. The experimental work for this study was made possible by a financial contribution from the California Committee on the Relation of Electricity to Agriculture.

[2] The authors are indebted to the members of the staff of the Division of Poultry Husbandry of the College of Agriculture, University of California, for cooperation in supplying suitable eggs for hatching and for the use of miscellaneous items of equipment. The samples of food and excreta were analyzed for nitrogen, carbon, and energy content by A. J. Soderberg.

[3] Italic numbers in parentheses refer to Literature Cited, p. 544.

[4] Cal. as used in this paper=kilogram-calorie.

The work of Kleiber and Dougherty (*11*) at temperatures ranging from 21° to 40° C. gave rise to questions as to the possibility of chicks surviving at temperatures below 21° and as to the relationship between heat production and energy intake at such low temperatures. The present series of trials was carried out to obtain information on these points. It was planned first to conduct trials at temperatures within the range studied by Kleiber and Dougherty for comparison and then to carry out trials at temperatures below this range to determine the lowest environmental temperature at which chicks would be able to grow. In particular, an attempt was made to determine whether there exists a temperature at which growth becomes negligible because all available energy that a chick can take in is used to produce heat for the maintenance of body temperature. In accordance with this plan, chicks were raised in environments the temperatures of which were within the range 21° to 38° C. and at 16° and 18°.

METHODS

The methods employed were similar to those described by Kleiber and Dougherty (*11*). Ten control birds were used, and five individuals were chosen for each experimental group. The chicks were hatched from trap-nested eggs from a flock in which the males were full brothers. Each individual in the experimental group was represented by two individuals from the same hen in the control group, except in the trial at 35° C., where this was true except in the case of one experimental bird, and in the trial at 18° where each experimental bird was matched with a half brother or sister and with one other individual. Control birds with body weights matching as closely as possible those of the corresponding experimental individuals were chosen.

At temperatures of 21° C. and above, the duration of each trial was 10½ days at full feed, followed by a 36-hour period during which the chicks had free access to water but received no food. The period of fast included the last 12 hours (night) of the twelfth day of each trial during which period fasting katabolism was determined. During the determination of the fasting katabolism little or no activity was observed. The duration of the trials at 16° and 18° was shortened by the failure of some individuals to survive at these temperatures. No fasting katabolism determinations could be made at 16° and 18° because the chicks which lived longer than 10 days were too weak to survive the long period of fasting.

The chicks were allowed free access to food, which was considered adequate for every requirement. The food, prepared in pellet form, contained:

25 parts of ground whole wheat.
25 parts of ground whole yellow corn.
25 parts of ground whole barley.
15 parts of fish scrap with 65 percent protein.
5 parts of dry skim milk.
5 parts of ground bone.

To 100 parts of this mixture were added:

2 parts of pulverized limestone (96 percent $CaCO_3$).
½ part of salt (NaCl).
1 part of cod-liver oil.
1 part of yeast.

Except for the yeast the ration used was identical with that used by Kleiber and Dougherty.

Lemon juice was added to the drinking water in line with the recommendation of Holst and Halbrook (*7*). The discovery of the fat-soluble antihemorrhagic vitamin (Almquist (*1*)) indicates that vitamin C probably was not necessary to the diet. Food intake was governed by the birds' appetite. Appetite in relation to environmental temperature, energy utilization, and rates of growth was thus studied. Kleiber and Dougherty *(11, p. 704)* have discussed appetite as "an important variable which determines in a great many cases the actual level of energy transformation in animals."

The experimental chicks were kept in the climatic cabinet described by Kleiber and Dougherty (*11*). The temperature was maintained automatically and seldom varied by as much as 2° C. from the desired level. Relative humidity was automatically maintained at 50 percent.

Air samples taken at the beginning and end of each (10- to 13-hour) period were analyzed in a modified Haldane apparatus (Kleiber (*9*)).

RESULTS

MORTALITY OF CHICKS

The limited number of chicks does not allow definite quantitative conclusions with respect to mortality at the various temperatures, yet the results indicate that the lowest limit of environmental temperature for these chicks was approached in the experiments.

None of the control chicks and none of the experimental birds kept at 21° C. or above died during these trials. At 18° one chick out of five died on the fifth day and one on the tenth day of the trial. The surviving chicks were removed from the chamber on the morning of the eleventh day of the trial. Of five chicks kept at 16°, one died on the sixth, one on the ninth, and one on the tenth day of the trial. The surviving chicks were removed from the chamber on the tenth day.

The chicks that were lost at 16° and 18° C. exhibited (before death) a dropsical condition of the foot and shank accompanied by redness of the skin of the leg and foot. During the last 2 days of life the droppings were sticky. True diarrhea such as that reported by Hall (*6*), who produced diarrhea in chicks by chilling them under a fan for short periods, was not observed. About 24 hours before death the chicks became very weak and failed to eat. On post-mortem examination it was found that the alimentary tract was entirely filled with food in each case. No abnormalties of the internal organs were observed.

The death rate probably was not influenced positively by the tendency of chicks to crowd together at low environmental temperatures, for only five birds were placed in the cabinet at a time. Under the conditions of the experiment huddling together actually may have resulted in a decrease of the death rate since it is a means of saving energy, as Kleiber and Winchester (*12*) have shown.

EFFECT OF TEMPERATURE ON THE GROWTH RATE OF CHICKS

Chicks hatched late in the season were noticeably smaller than those hatched earlier. To avoid any influence on the results that might have been exerted by seasonal variations in the birds, the mean daily increase in weight of the experimental chicks was calculated as percentage of the mean daily increase of the corresponding controls. The result of this calculation expressed as a curve is given in figure 1.

This curve shows relative rates of growth through a period of 8 days. The rate of increase in weight per day was 2.2 ± 0.4 g [5] for the chicks kept at 16° C., while the mean rate of increase for the experimental chicks kept at temperatures higher than 16° (18° to 38°, inclusive) was 3.9 ± 0.3 g per day. That of all the control birds was 4.4 ± 0.3 g per day. Growth rate data are given in table 1.

Growth rate was slowest at 16°, and most rapid at 21° C. as is shown by the next to the last column in table 1. Kleiber and Dougherty (*11*) observed that the rate of growth increased as environmental

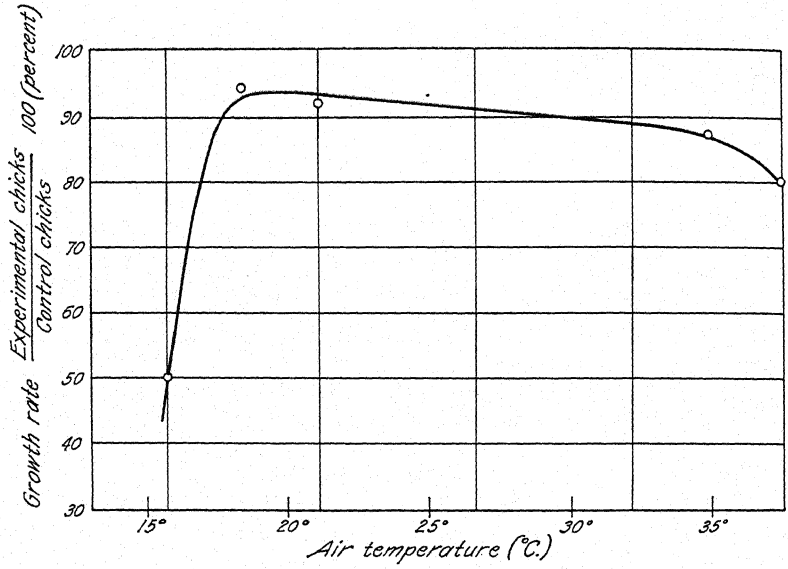


FIGURE 1.—Mean daily increase in weight of chicks kept at different temperatures, expressed as percentage of mean daily increase of corresponding controls. Rates of growth shown during a period of 8 days.

temperature was decreased to 21°, which parallels the results of the present experiment in a corresponding temperature range. That growth took place at all at 16° is a rather remarkable fact. The manner in which energy was spared for growth at this (comparatively) low temperature will be discussed later.

TABLE 1.—*Weight and growth-rate data for chicks kept at different temperatures*

Environmental temperature (°C.)	Experimental groups				
	Mean weight of chicks at start of trial	Mean weight of chicks at end of 8 days	Mean weight of chicks at end of 11 days	Mean daily increase through period of 8 days	Mean daily increase through period of 11 days
	Grams	*Grams*	*Grams*	*Grams*	*Grams*
38	35.5±0.9	58.0±1.3	69.4±1.6	2.8±0.2	3.1±0.2
35	50.1± .8	86.1±2.5	102.5±3.4	4.5± .3	4.8± .4
21	45.0±1.3	77.2±2.3	99.2±3.3	4.0± .3	4.9± .3
18	47.9±1.4	83.2±2.3	----------	4.4± .3	----------
16	43.8±1.0	61.1±2.8	----------	2.2± .4	----------

[5] Throughout this paper the figure following the sign refers to the standard error of the mean.

TABLE 1.—*Weight and growth-rate data for chicks kept at different temperatures*—Continued

Environmental temperature (°C.)	Control groups					Daily increase of experimental chicks expressed as percentage of daily increase of control chicks at ages shown	
	Mean weight of chicks at start of trial	Mean weight of chicks at end of 8 days	Mean weight of chicks at end of 11 days	Mean daily increase through period of 8 days	Mean daily increase through period of 11 days	8-day period	11-day period
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*
38	34.9±1.5	63.0±1.4	78.5±1.9	3.5±0.3	4.0±0.2	80.0	77.5
35	50.2±1.1	91.2±2.4	109.0± .7	5.1± .3	5.3± .5	88.2	90.6
21	44.9± .9	79.7±2.0	102.8±1.9	4.3± .3	5.3± .2	93.0	92.4
18	47.7± .9	86.3±2.3	----------	4.8± .3	----------	91.7	----------
16	43.8±1.0	78.3±1.7	----------	4.3± .2	----------	51.2	----------

A formula for determining the relative rate of growth during the accelerating phase of growth has been given by Brody (*4*). The formula is as follows:

$$\frac{dW}{dt}=kW$$

where

W=*body weight in grams*
t=time in days

The integrated form has been used for these calculations, namely:

$$k=\frac{lnW_2-lnW_1}{t_2-t_1}=2.30259\ \frac{\log\ W_2-\log\ W_1}{t_2-t_1}$$

The calculations were based on the weights obtained the morning of the fifth day of life and those obtained on the morning of the ninth, the thirteenth, and the sixteenth day. The results of these calculations are given in table 2. The relative rate of growth at 16° C. was distinctly lower than that at any higher temperature.

The growth rates of chicks raised at 16° and 18° C. are given for the first 8 days of the trial only, since some chicks kept at the two lower temperatures did not survive longer.

TABLE 2.—*Relative growth rate of chicks of various ages, kept at different temperatures as determined by Brody's formula:*

$$k=2.30259\frac{(\log\ W_2-\log\ W_1)}{(t_2-t_1)}$$

Air temperature (°C.)	Percent growth per day: (100 k) during period of age indicated				
	5–8 days (inclusive)	9–12 days (inclusive)	13–15 days (inclusive)	5–12 days (inclusive)	5–15 days (inclusive)
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
16	2.6	5.8	----------	4.2	----------
18	6.1	7.7	----------	6.9	----------
21	5.9	7.6	8.4	6.8	7.2
35	7.5	6.1	5.8	6.8	6.5
38	5.5	6.7	6.0	6.1	6.1

EFFECT OF TEMPERATURE ON FOOD CONSUMPTION, RATE OF EXCRETION, AND AVAILABILITY OF NUTRIENTS

During the period from the fifth to the twelfth days, inclusive, (during which it was possible to make comparisons of chicks at all the temperatures used) the food consumption per chick was greatest at 18° C. and least at 38°. Because of the variations in weight of the birds, a better comparison can be made in terms of body size. Kleiber (*8*) has found the 0.75 power of the body weight to be the most suitable means of comparing the metabolism of animals of various sizes. Brody and Procter (*5*) found the 0.73 power of the body weight to be best for making comparisons of metabolism. The 0.75 power of the body weight is used throughout this paper for comparing the body weight of chicks. The symbol $kg^{3/4}$ is used here as the unit of the 0.75 power of the body weight expressed in kilograms.

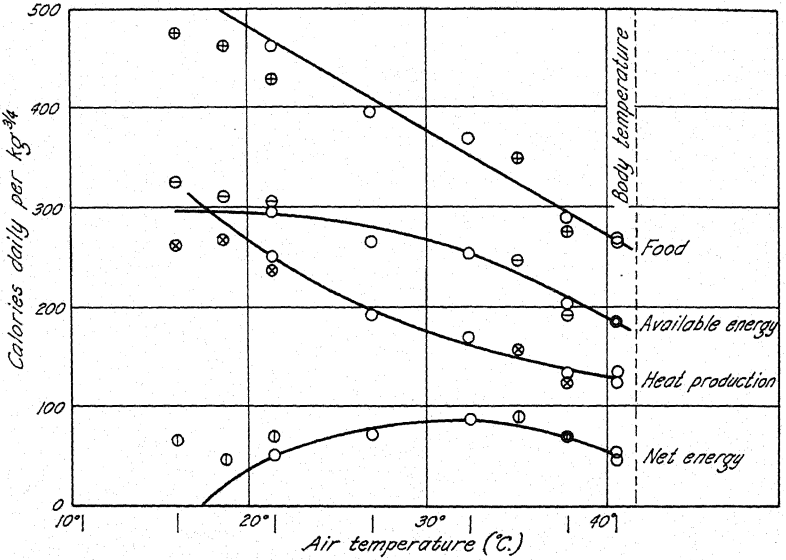


FIGURE 2.—Rate of food consumption and heat production by, and net and available energy of food of chicks as related to environmental temperature. Curves are those of Kleiber and Dougherty (*11, p. 717*). The original points are represented by circles; corresponding data from the present experiment are indicated by circles enclosing crosses, and horizontal and vertical lines.

The chicks kept in an environment of 16° C. consumed more food per $kg^{3/4}$ than those kept at higher temperatures. The relation of food consumption and environmental temperature seems to be nearly linear, as figure 2 shows.

Availability of food was somewhat greater at 21°, 35°, and 38° C. than at 16° and 18° (table 3). The term "availability" as employed here means the amount of dry matter retained by the body, expressed as percentage of the dry matter consumed. The dry matter not excreted is "available" either for production of body substance or for production of heat. This conception is very useful in nutrition work with chicks where *digestibility* of food would be difficult to determine owing to the fact that feces and urine of birds cannot easily be sep-

arated. The results for the various periods of each trial agree quite closely.

The availability for the period including the fifth and twelfth days is plotted against the environmental temperatures in figure 3. At 35° and 38° C. the availability was very similar to that determined by Kleiber and Dougherty, who found the maximum at 38°; but in the present

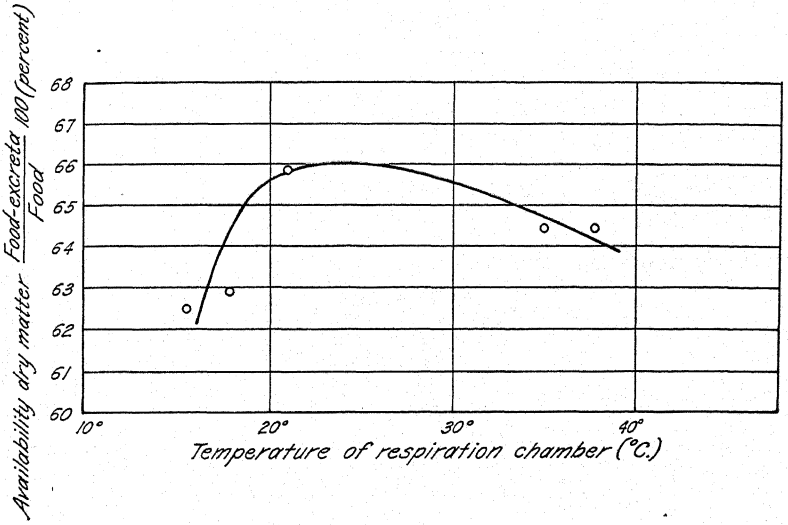


FIGURE 3.—Availability of food dry matter to chicks as influenced by environmental temperature.

experiment maximum availability of nearly 66 percent was found at 21°, while availability at 16° and at 18° was approximately 63 percent as compared with more than 64 percent at 35° and 38°, during the fifth to twelfth day period.

TABLE 3.—*Food consumption, excretion, and food availability per day for chicks of various ages kept at different temperatures, expressed on a dry-matter basis*

Age (days)	Item	Data for indicated air temperature				
		16° C.	18° C.	21° C.	35° C.	38° C.
5–8	Food—grams	9.67	10.78	9.73	10.11	5.64
	Excretion—do	3.65	4.10	3.47	3.51	2.05
	Available—do	6.02	6.68	6.26	6.60	3.59
	Availability—percent	62.30	62.00	64.30	65.30	63.70
9–12	Food—grams	14.79	16.74	15.10	11.86	7.56
	Excretion—do	5.49	6.10	5.00	4.30	2.65
	Available—do	9.30	10.64	10.10	7.56	4.91
	Availability—percent	62.90	63.60	66.90	63.70	65.00
13–15	Food—grams			20.15	12.67	9.27
	Excretion—do			6.67	4.24	3.46
	Available—do			13.48	8.43	5.81
	Availability—percent			66.90	66.50	62.70
5–12	Food—grams	12.23	13.76	12.42	10.99	6.60
	Excretion—do	4.57	5.10	4.24	3.91	2.35
	Available—do	7.66	8.66	8.18	7.08	4.25
	Availability—percent	62.60	62.90	65.90	64.40	64.40
5–15	Food—grams			14.53	11.45	7.33
	Excretion—do			4.99	4.00	2.65
	Available—do			9.54	7.45	4.68
	Availability—percent			65.70	65.10	63.90

Considering the fact that the digestive systems of the birds at the two lower temperatures were probably taxed to full capacity, an availability of 63 percent at 16° and 18° C. seems quite favorable as compared with availabilities of 64 to 66 percent observed at more nearly optimal temperatures. The ration used was as nearly as possible equal to that fed by Kleiber and Dougherty except for the addition of yeast to the feed and lemon juice to the drinking water. The greatest difference between the availability observed in this trial and that of the authors mentioned was 8 percent at 21°.

RESPIRATORY EXCHANGE OF CHICKS AS AFFECTED BY TEMPERATURE

The metabolism of chicks apparently tended to increase as environmental temperature was lowered to 18° C. This result parallels the observations of Kleiber and Dougherty. The carbon dioxide production per $kg^{3/4}$ is larger in the present series of trials than in the earlier series at each environmental temperature. Maximum CO_2 per $kg^{3/4}$ was produced at 18° during the first 8 days of the trial (table 4). At each level of temperature above 18° C. the carbon dioxide production was successively lower. At 16° the CO_2 production was less than that at 18°.

TABLE 4.—*Carbon dioxide produced per day by chicks of various ages kept at different temperatures*

Basis, period, and age of chicks	Data for indicated environmental temperatures				
	16° C.	18° C.	21° C.	35° C.	38° C.
Per chick	*Liters*	*Liters*	*Liters*	*Liters*	*Liters*
Period 1 (5–8 days)	4.87	5.55	4.61	3.95	2.20
Period 2 (9–12 days)	6.31	7.32	6.67	4.62	2.81
Period 3 (13–15 days)			8.42	6.09	3.49
5–12 days	5.59	6.44	5.64	4.29	2.54
5–15 days			6.40	4.78	2.75
Per kilogram of body weight [1]					
Period 1 (5–8 days)	105.1	108.2	93.3	71.0	54.7
Period 2 (9–12 days)	93.2	106.6	103.9	62.4	57.6
Period 3 (13–15 days)			99.6	65.8	58.1
5–12 days	97.9	107.3	99.3	66.2	56.3
5–15 days			99.4	66.0	56.9
Per $kg^{3/4}$					
Period 1 (5–8 days)	48.7	51.4	43.9	34.3	24.3
Period 2 (9–12 days)	47.1	54.2	52.5	32.5	26.8
Period 3 (13–15 days)			53.3	36.2	28.8
5–12 days	47.8	52.8	48.2	33.5	25.9
5–15 days			50.4	33.7	26.7

[1] Average of mean daily body weights.

TABLE 5.—*Respiratory quotients of full-fed chicks of various ages kept at different temperatures*

Age of chicks (days)	Respiratory quotients for indicated environmental temperature					
	16° C.		18° C.		35° C.	
	Day	Night	Day	Night	Day	Night
5–8	1.005	0.830	0.961	0.843	0.996	0.990
9–12	1.001	.812	.931	.820	1.029	.972
5–12	1.003	.820	.950	.831	1.012	.981
Day and night	0.912		0.889		0.998	

The respiratory exchange at 18° C. during the first 8 days was slightly more than twice that at 38°. Respiratory quotients of full-fed chicks are given in table 5 for the trials at 16°, 18°, and 35°.[6] Kleiber and Dougherty (*11*, pp. 714–715) have stated that "there seems to be a tendency for the R. Q. to be lower at the extremely low and high air temperatures." This tendency appears to have been confirmed for low temperatures in the present experiment, as the respiratory quotients at 16° and 18° were 0.912 and 0.889, respectively, while the lowest respiratory quotient reported in the experiment just cited was 0.971 (at 21°). At 35° the respiratory quotient (in the present trials) was 0.998, which is quite close to an average of the respiratory quotients reported earlier (*11*) for 32° and 38°. Marked variations between day and night respiratory quotients similar to those reported earlier were observed. Kleiber and Dougherty assume that the lower respiratory quotient found at night was a result of decreased fat production of the birds during the hours that they were without food. A comparison of the respiratory quotients in table 5 with those in table 7 shows the respiratory quotients at full feed to be much higher than those of fasting chicks.

NITROGEN, CARBON, AND ENERGY BALANCE

The excreta and food were analyzed for nitrogen, carbon, and energy content. The quantities of dry matter ingested and excreted daily were determined. The differences between the amounts of nitrogen, carbon, and energy ingested and those excreted are the quantities available for production of body substance or for combustion. For each liter of carbon dioxide given off by the animal 0.5359 g of carbon was subtracted from the total carbon available. The balance is considered as the net carbon or the carbon stored in the body. To find the amount of protein gained, the figure for available nitrogen was multiplied by 6.25 and to find the amount of carbon in the protein gained, the amount of available nitrogen was multiplied by 3.25. The amount of carbon in the body fat produced during the trial was found by subtracting the amount of carbon in the protein from the total amount of carbon stored. The net energy[7] gained was found by obtaining the sum of the heat of combustion of stored fat and of protein gained.[8] The difference between the available energy intake and net energy gained was considered equivalent to the heat production of the animal.

Data on energy metabolism are given in table 6. Since some of the chicks held at 16° and 18° C. died during the first few days of the trials, the results for these trials are given only for the period from 5 to 12 days inclusive, and, for purposes of comparison, data for the first 8 days of the other trials are given, as well as the data obtained during the 11-day period when chicks at temperatures of 21° and above were at full feed.

The hypothesis of Kleiber and Dougherty that "with lower temperature, the heat production tends to approach the energy intake, since the latter is naturally limited," seems to be confirmed by the results

[6] At temperatures of 21° and 38° C. oxygen consumption was measured only during the fasting katabolism determinations.

[7] As used here the term "net energy" or "net energy in production" refers to energy in body substance gained.

[8] 1 g of protein=5.7 Cal. (kilogram-calories).
1 g of fat =9.5 Cal. (kilogram-calories).

of the present trials. During the first 8 days of the trial at 38° C., 44 percent of the energy taken in was used to produce heat; at 35°, 45 percent; at 21°, 55 percent; and at 18°, 58 percent. The heat production per $kg^{3/4}$ was greater with each decrease in environmental temperature, including the trial conducted at 18°. At 16°, however, the heat production was 261.7 Cal. as compared with 266.1 Cal. per $kg^{3/4}$ at 18°. It is difficult to account for a lower rate of heat production at 16° than at 18° unless it be assumed that the mean body temperature was lower at 16° than at 18°. This assumption would also help to explain the surprising fact that net energy in production was found to be greater at 16° than at 18°. At 16° net energy in production was 63.5 Cal. per $kg^{3/4}$, while at 18° it was 45.7 Cal. per $kg^{3/4}$. During the first 8 days of the trials the mean net energy in production was 75.7 Cal. per $kg.^{3/4}$ at 21°, 35°, and 38°; and for the entire 11-day period the corresponding figure was 74.2. The only record that the writers have found of reduced body temperature in birds at low environmental temperature is that of Benedict and his coworkers (*3*), who found that the mean rectal temperatures of a group of mature homozygous frizzle fowl, determined during a period of 3 days at environmental temperatures ranging from 5.6° to 13.9°, was 41.2° as compared with 41.6° for a group of normal chickens under the same conditions.

TABLE 6.—*Energy transformation at full feed, and composition and energy content of the daily gain of body weight, for chicks kept at different environmental temperatures while 5 to 12 and 5 to 15 days old*

Item	Data for indicated environmental temperature and age of chicks							
	16° C., 5 to 12 days	18° C., 5 to 12 days	21° C.		35° C.		38° C.	
			5 to 12 days	5 to 15 days	5 to 12 days	5 to 15 days	5 to 12 days	5 to 15 days
Mean body weight, grams	52.4	65.5	61.1	72.1	68.1	76.3	46.7	52.4
Mean body size, $kg^{3/4}$	.110	.130	.123	.140	.133	.145	.102	.110
Energy in food per day per $kg^{3/4}$, Calories	472.7	461.3	429.4	441.3	351.3	335.8	275.1	283.3
Energy in excreta per day per $kg^{3/4}$, Calories	147.5	149.5	122.3	126.5	104.2	97.9	84.2	88.2
Available energy per day per $kg^{3/4}$, Calories	325.2	311.8	307.1	314.8	247.1	237.9	190.9	195.1
Heat production per day per $kg^{3/4}$, Calories	261.7	266.1	237.9	235.4	158.8	168.3	121.3	121.4
Energy in gained body substance per day per $kg^{3/4}$ Calories	63.5	45.7	69.2	79.4	88.3	69.6	69.6	73.7
Daily gain in body weight grams	2.16	4.41	4.03	4.93	4.51	4.76	2.81	3.08
Daily production of body protein grams	.81	1.06	1.06	1.25	1.06	1.19	.63	.69
Daily production of body fat do	.25	−.01	.26	.42	.60	.35	.37	.44
Energy in body substance gained daily Calories	6.99	5.94	8.51	11.12	11.74	10.10	7.10	8.11
Body protein per gram increase in body weight grams	.375	.240	.263	.254	.235	.250	.224	.224
Body fat per gram increase in body weight grams	.116	−.003	.065	.085	.133	.074	.132	.143
Inorganic matter per gram increase in body weight (mostly water) grams	.509	.762	.672	.661	.632	.676	.644	.633
Energy per gram increase in body weight Calories	3.24	1.35	2.11	2.26	2.60	2.12	2.53	2.63

Figure 2 represents the relationship between the temperature of the air and the energy transformations in the birds' bodies. The energy exchange is calculated per $kg^{3/4}$ in order to avoid any possible influence of body size. The curves given in figure 2 are reproduced from the work of Kleiber and Dougherty (*11*), and because the data obtained in the present experiment confirm the earlier work insofar as the higher temperatures are concerned, new curves have not been fitted. At 16° and 18° C. the extrapolations of Kleiber and Dougherty do not seem to fit the data obtained in the present experiment, which can be explained by the fact that the extrapolations were based on the assumption that body temperature and specific insulation of the chicks would remain constant. As has been mentioned, it is assumed that the mean body temperature of the chicks was reduced at a low environmental temperature, for otherwise these results could not easily be explained.

COMPOSITION AND ENERGY CONTENT OF SUBSTANCE GAINED BY BODIES OF CHICKS

The effects of environmental temperatures on the daily production of protein, fat, and net energy are shown in table 6. Carbon and nitrogen balances were used in calculating the energy metabolism of the chicks on the assumption that the organic constituents of the body substance gained were mainly protein and fat. For the 8-day period there was no production of body fat at 18° C. Fat storage at 16° was approximately equal to that of 21°, but less than that at 35° and 38° during the first 8 days of the trial.

The daily gain of protein per chick was 1.06 g during the first 8 days of the trial at 18°, 21°, and 35° C. At 16° where the daily gain of body weight was markedly lower, daily gain of protein was 0.81 g. At 38°, where the daily gain in weight was also comparatively low, the gain in protein was 0.63 g. Except at 16° the gain in protein per gram increase in body weight ranged only from 0.22 g at 38° to 0.26 g at 21°. Kleiber and Dougherty (*11*) found that the gain in protein changed only from 0.22 to 0.27 g per gram of increase in body weight at temperatures ranging from 21° to 40°. At 16° in the present series of trials 0.37 g of protein was gained per gram of body substance gained.

At 16° C. 7 Cal. of net energy was gained daily, while at 18°, where the daily gain in body weight was more than twice that at 16°, the daily gain in net energy was approximately 6 Cal. This seems to confirm the statement that "the body weight is no general criterion for the effect of food on energy storage in the animal." At 18° water and other inorganic materials constituted 76.2 percent of the gain in body weight, while at 21° the content of inorganic material was 67.2 percent during the same period of the trial (fifth to twelfth days inclusive). At 16° the water content of body substance gained was 50.9 percent. This was far lower than the water content of body substance gained at any higher temperature.

FASTING KATABOLISM

Rates of fasting katabolism were determined over a period of approximately 12 hours during the night. The chicks were fasted 24 hours before the beginning of these determinations. Since the chicks

kept at 16° and 18° C. were too weak to survive a 36-hour fast, no determinations of fasting katabolism could be made of these two groups. Rates of fasting katabolism of the birds kept at 21°, 35°, and 38° are given in table 7. These results are calculated to a 24-hour basis. A standard of 4.7 Cal. per liter of oxygen consumed was used to determine the heat production of the chicks.

TABLE 7.—*Fasting katabolism (calculated to 24-hour basis) of chicks at 16 days of age, when kept at different temperatures*

Air temperature (°C.)	Body weight	$kg^{3/4}$	Daily respiratory exchange per chick calculated to 24 hours		Respiratory quotient	Daily heat production		
			CO_2	O_2		Per chick	Per kilogram	Per $kg^{3/4}$
	Grams		*Liters*	*Liters*		*Calories*	*Calories*	*Calories*
21	84.2	0.156	3.70	5.24	0.706	24.6	292	158
35	95.7	.172	2.58	3.59	.719	16.9	177	98
38	62.5	.125	1.69	2.32	.728	10.9	174	87

Daily heat production as determined in this series of trials was somewhat greater than that reported by Kleiber and Dougherty (*11*). At 21° C. the daily heat production per $kg^{\frac{3}{4}}$ was 158 Cal., whereas Kleiber and Dougherty found daily heat production to be 128 Cal. at this temperature. Similarly, daily heat production at 35° and 38° was 98 and 87 Cal. respectively. Kleiber and Dougherty found daily heat production to be 90 Cal. at 32° and 83 Cal. at 38°.

CALORIGENIC ACTION OF FOOD AT VARIOUS ENVIRONMENTAL TEMPERATURES

In calculating calorigenic action the figures for metabolism at full feed and for fasting katabolism were used. Since it was impossible to obtain data on the fasting katabolism of birds raised at 16° and 18° C., the heat increment was calculated only for birds raised at environmental temperatures of 21°, 35°, and 38°. These data are given in table 8.

TABLE 8.—*Calorigenic action of food of chicks kept at different environmental temperatures*

Item	Data for indicated environmental temperatures		
	21° C.	35° C.	38° C.
Daily basal heat production per $kg^{3/4}$ calculated to the age of 10 days	149	92	82
Daily heat production at full feed per $kg^{3/4}$ ..... Calories	235	168	121
Daily heat increment at full feed per $kg^{3/4}$ ..... do	86	76	39
Daily available energy in food per $kg^{3/4}$ ..... do	315	238	195
Heat increment per 100 Cal. available energy at full feed ..... percent	27	32	20

Since the average age at full feed was 10 days, and the age at which the fasting katabolism was determined was 16 days, it was necessary to calculate a theoretical fasting katabolism for the age of 10 days.

The fact that the metabolism at full feed bears a close relationship to the energy value of food consumed, and the observation of Kleiber (*10*) that for very different animals the total energy intake seems to be approximately equal to the same multiple of the fasting katabolism, make possible the calculation of the calorigenic action. It is assumed that the fasting katabolism of the birds, during the period studied, increased by 1 percent of the mean for each additional day of age as determined for the metabolism at full feed.

The following equation was used to calculate a theoretical fasting katabolism for chicks 10 days old (*11*): [9]

$$B_{10}\times(1+0.01\times 6)=B_{16}$$

where

B_{10}=fasting katabolism per $kg^{\frac{3}{4}}$ at the age of 10 days

B_{16}=fasting katabolism per $kg^{\frac{3}{4}}$ at the age of 16 days.

EFFICIENCY OF ENERGY TRANSFORMATION AT VARIOUS TEMPERATURES

The terms "partial efficiency" and "total efficiency" are employed in the same sense as they are used by Kleiber and Dougherty (*11*). The "partial efficiency" can be represented as a quotient obtained by dividing the figure representing an increment of net energy by that for the energy intake necessary to produce the increment of net energy.

$$p=\frac{\Delta A}{\Delta U}$$

p=partial efficiency

where

ΔA=change in net energy

ΔU=corresponding change in energy intake

In this equation ΔA may mean an increase in energy in the animal's body when a trial is carried out above the maintenance level, or it may mean a decrease in the loss of energy resulting from an increase of food consumption in the case of a trial carried out below the maintenance level. Its value is positive in either case. The increase of net energy has been calculated as the difference between the amount of available food energy taken in and the corresponding heat increment (availability is defined on page 534). Thus the partial efficiency at three environmental temperatures has been calculated from the data in table 8. The partial efficiency, in terms of net energy per 100 Cal. of available food energy is, for temperatures of 21°, 35°, and 38° C., 73, 68, and 80 Cal., respectively. Since it was impossible to obtain data on the basal metabolism of chicks at air temperatures of 16° and 18° the partial efficiency for these groups cannot be given. As will be noticed in table 8, there is a considerable difference in the heat increment at 35° and 38°, a discrepancy for which no explanation is now available.

[9] Experiments are under way from which it is hoped to measure this relation of age to basal metabolism. It is seen from the equation above that the correction for age for the present trials amounts to 6 percent of the fasting katabolism. Even if the coefficients of age were actually considerably different from that assumed (1 percent) the total correction would not seriously affect the conclusions reported here.

The term "total efficiency" as used here refers to the net energy expressed as percentage of the available food energy. The total efficiency and the partial efficiency are related (*11*), as is shown by the following equation.

$$\eta_t = \frac{A}{U} = \frac{\eta_p (U-E)}{U} = \eta_p \left(1 - \frac{E}{U}\right)$$

where

η_t = total efficiency
η_p = partial efficiency
U = energy of food intake
E = energy derived from food for maintenance
A = net energy in production

The total efficiency would approach the partial efficiency if a very small proportion of the total food energy were required for maintenance, and it equals zero when the energy necessary for maintenance becomes equal to total food energy taken in.

Fasting heat production data have been used in calculating the partial efficiency, but they are not necessary for calculating the total efficiency; therefore the figures for total efficiency cannot have been influenced by possible errors which might be present in the measurement of partial efficiency such as the effect of social temperature regulation on the fasting heat production. The total efficiency calculated from data in table 6 is presented in table 9. The lowest total efficiency was found at 18° C., while the total efficiency at 16° was higher than that at 18° but not so high as that at 21° during the first 8 days of trial. Through an experimental period of 8 days the greatest total efficiency was found at 35° and 38° (36 percent).

TABLE 9.—*Total efficiency of energy utilization in growing chicks kept at different temperatures while 5 to 12 and 5 to 15 days old*

Item	Total efficiency for indicated environmental temperature and age of chicks							
	16° C.	18° C.	21° C.		35° C.		38° C.	
	5–12 days	5–12 days	5–12 days	5–15 days	5–12 days	5–15 days	5–12 days	5–15 days
Energy in gained body substance ×100 / Available food energy	*Percent* 20	*Percent* 15	*Percent* 23	*Percent* 25	*Percent* 36	*Percent* 29	*Percent* 36	*Percen* 38

SUMMARY

White Leghorn chicks were raised at environmental temperatures of 16°, 18°, 21°, 35°, and 38° C. from 5 to 14, 15 or 16 days of age, inclusive. Groups of five chicks were kept at controlled air temperatures and were matched by groups of 10 chicks kept in a conventional brooder. Both the control and the experimental groups had free access to feed which was considered qualitatively complete. The respiratory exchange of the chicks kept at controlled air temperatures was measured, and the fasting katabolism was determined. Food

consumed and excreta were weighed and were analyzed for nitrogen, carbon, and energy content.

No deaths occurred among the chicks kept at 21° C. or above, nor among the control birds. Of five chicks kept at 18° two had died by the end of the tenth day of the experiment, and of the same number kept at 16° three had died.

Post-mortem examination of the birds lost at 16° and 18° C. disclosed no observable abnormalities of the internal organs, but in each the alimentary tract was entirely filled with food.

The mean growth rate of the control birds was 4.4 ± 0.3 g per day, while that of the experimental chicks ranged from 2.2 ± 0.4 g at 16° C. to 4.5 ± 0.3 g at 35° during the first 8 days of the experiment.

Food consumed was approximately a linear negative function of the environmental temperature.

Availability of food, or $\frac{\text{food} - \text{excreta}}{\text{food}} \times 100$, was somewhat the greater at higher temperatures than at 16° and 18° C.

The CO_2 production reached a maximum at 18° C., and decreased as environmental temperature increased. CO_2 production per $kg^{3/4}$ (0.75 power of body weight expressed in kilograms) was less at 16° than at 18°.

Energy of body substance gained per day exhibited a minimum of 45.7 Cal. per $kg^{3/4}$ at 18° C., and a maximum of 88.3 Cal. per $kg^{3/4}$ at 35° during the first 8 days of the experiment. At 16°, 63.5 Cal. of net energy was stored as gain in body substance per $kg^{3/4}$ per day.

The environmental temperature had a pronounced effect on the composition of body substance gained. The amount of fat stored per gram of increase in body weight was greatest at 35° and 38° C. At 18° no fat was stored, while at 16° fat storage was exceeded only by that at 35° and 38° during the first 8 days of the experiment. The gain of protein per gram increase in body weight was greatest at the lowest environmental temperature. The absolute increase in protein at 16° was exceeded at all the higher temperatures except 38°. The gain in protein varied from 0.224 g (38°) to 0.375 g (16°) per gram of increase in body weight. Maximum energy content per gram of weight gained was 3.24 Cal. at 16°, and the minimum per gram of weight gained was 1.35 Cal. at 18°. The minimum amount of water (0.509 g per gram increase in body weight) was stored at 16°; the maximum (0.762 g per gram increase in body weight) was stored at 18°.

The following fasting katabolic rates were determined: 158 Cal. per $kg^{3/4}$ per day at 21° C., 98 Cal. per $kg^{3/4}$ per day at 35°, and 87 Cal. per $kg^{3/4}$ per day at 38°.

The partial efficiency, or the increase in net energy per unit of the corresponding increase in food energy, was determined for environmental temperatures of 21°, 35°, and 38° C.

At 35° and 38° C. total efficiency, or total energy in the produced body substance expressed as percentage of the available food energy taken in, was 36 percent during the first 8 days of the experiment, while at 21° total net energy was 23 percent of the available energy. At 18° net energy was 15 percent of the available energy, and at 16° it was 20 percent.

LITERATURE CITED

(1) ALMQUIST, H. J.
1937. THE ANTI-HEMORRHAGIC VITAMIN. Poultry Sci. 16: 166–172.
(2) BAROTT, H. G., BYERLY, T. C., and PRINGLE, EMMA M.
1936. ENERGY AND GASEOUS METABOLISM OF NORMAL AND DEUTECTOMIZED CHICKS BETWEEN 10 HOURS AND 100 HOURS OF AGE. Jour. Nutrition 11: 191–210, illus.
(3) BENEDICT, FRANCIS G., LANDAUER, WALTER, and FOX, EDWARD L.
1932. THE PHYSIOLOGY OF NORMAL AND FRIZZLE FOWL, WITH SPECIAL REFERENCE TO THE BASAL METABOLISM. Conn. (Storrs) Agr. Expt. Sta. Bull. 177, pp. [13]–101, illus.
(4) BRODY, SAMUEL.
1927. TIME RELATIONS OF GROWTH. III. GROWTH CONSTANTS DURING THE SELF-ACCELERATING PHASE OF GROWTH. Jour. Gen. Physiol. 10: 637–658, illus.
(5) ——— and PROCTOR, R. C.
1932. GROWTH AND DEVELOPMENT WITH SPECIAL REFERENCE TO ANIMALS. XXIII. RELATION BETWEEN BASAL METABOLISM AND MATURE BODY WEIGHT IN DIFFERENT SPECIES OF MAMMALS AND BIRDS. Mo. Agr. Expt. Sta. Research Bull. 166: 89–101, illus.
(6) HALL, G. E.
1932. EXTERNAL TEMPERATURE AS A FACTOR IN THE PRODUCTION OF DIARRHEA IN YOUNG CHICKS. Poultry Sci. 11: 250–252.
(7) HOLST, WALTHER F., and HALBROOK, EVERETT R.
1933. A "SCURVY-LIKE" DISEASE IN CHICKS. Science 77: 354.
(8) KLEIBER, MAX.
1932. BODY SIZE AND METABOLISM. Hilgardia 6: [317]–353, illus.
(9) KLEIBER, M.
1933. CONTRIBUTION TO THE METHOD OF GAS ANALYSIS FOR RESPIRATION TRIALS. Jour. Biol. Chem. 101: 583–594, illus.
(10) ———
1933. TIERGRÖSSE UND FUTTERWERTUNG. Biedermanns Centbl. Agr. Chem. (Abt. B, Tierernährung) 5: 1–12. [Summaries in both German and English, pp. 11–12.]
(11) ——— and DOUGHERTY, J. E.
1934. THE INFLUENCE OF ENVIRONMENTAL TEMPERATURE ON THE UTILIZATION OF FOOD ENERGY IN BABY CHICKS. Jour. Gen. Physiol. 17: 701–726, illus.
(12) ——— and WINCHESTER, C. F.
1933. TEMPERATURE REGULATION IN BABY CHICKS. Soc. Expt. Biol. and Med. Proc. 31: 158–159.
(13) LEWIS, HARRY R.
1912. REPORT OF THE POULTRY HUSBANDMAN. N. J. Agr. Expt. Sta. Ann. Rept. (1911) 32: 89–155, illus.

SOME PHYSIOLOGICAL STUDIES OF CROWN GALL AND CONTIGUOUS TISSUE [1]

By R. NAGY, *industrial fellow in biochemistry and plant pathology;* A. J. RIKER, *professor of plant pathology;* and W. H. PETERSON, *professor of biochemistry, Wisconsin Agricultural Experiment Station* [2]

INTRODUCTION

As part of an investigation of the pathological plant growth induced by the crown gall organism it appeared desirable to study the nature and physiology of both diseased and contiguous tissue. Only a few comparisons of such tissues have been reported. Strohmer and Stift (*31*) [3] found that the galls of sugar beets were higher in ash, protein, and moisture but lower in sugar than the normal roots. Townsend (*34*) and others found that the galled beets were decidedly lower in sucrose than the normal ones. Klein and Keyssner (*15*) have made an extensive study of the forms of nitrogen found in galls and contiguous tissue of several hosts. In most instances the percentage of the various forms of nitrogen was higher in the galls than in the contiguous stems. Sylwester and Countryman (*32*) found both callus and gall tissue of apple to contain cellulose, pectin, lignin, and gum. The gall tissue also contained tannin, but the callus tissue did not. Berthelot and Amoureux (*4*) found similar differences in tannin content between galls and normal tissue of sugar beets. They (*3*) also compared gall tissues resulting from inoculation of sugar beets with two strains of *Phytomonas tumefaciens* (Smith and Town.) Bergey et al., but obtained no significant differences.

Glutathione and ascorbic acid have been considered to play an important role in the growth of plants. Hammett (*12*) in particular has emphasized the importance of glutathione. Through its constituent amino acids glutathione probably accelerates cell proliferation and protein reconstitution and differentiation. Virtanen (*36*) reported large increases in dry weight of plants grown in sterile nutrient solutions containing ascorbic acid. Binet and Magrou (*6*) and Berthelot and Amoureux (*4*) reported greater amounts of glutathione in crown gall than in the host plant. The latter investigators likewise found nearly twice as much ascorbic acid in the galls as in the beet root.

Several investigators have suggested a relation between the activities of oxidative enzymes and atypical growth. For example, Bristol (*7*) attributed cell stimulation to the unchecked action of locally concentrated, intercellular oxidizing enzymes. A disproportionate balance between the enzymes, especially an overabundance of peroxidase, he

[1] Received for publication March 17, 1938; issued October, 1938. This study was made possible by grants from the International Cancer Research Foundation and from the Wisconsin Alumni Research Foundation, University of Wisconsin.

[2] The writers were ably assisted with certain of the analyses by H. A. Conner and F. C. McIntire. The illustrations were prepared by Eugene Herrling.

[3] Italic numbers in parentheses refer to Literature Cited, p. 553.

postulated, would result in hyperoxidation in the epithelial cells. Klein and Ziese (*16*) found the catalase activity in crown gall of beets to be greatly increased over that in healthy beets. Likewise (*17*) an increase in peroxidase in crown gall of horseradish paralleled the increase in catalase. The activity of diseased tissue was 80 to 100 percent greater than that of healthy tissue. Smith (*28, 29*) and Robinson (*25*) were of the opinion that atypical growth may be caused by uncontrolled respiration but that the initial cell stimulation was caused by an "oxygen-hunger." In view of the strongly aerobic nature of *Phytomonas tumefaciens* (*26*), the oxygen may be greatly reduced in the intercellular spaces, thus modifying the enzyme relation and creating an environment for cell stimulation. According to Kluyver (*18, p. 529*), "* * * the conversion of a normal tissue cell into a cancer cell could ultimately depend on a quantitative change in property of one single catalytic agent which determines metabolism." In view of the importance of oxidative enzymes in cell metabolism it seemed desirable to compare the catalase, peroxidase, and oxidase content of normal and crown gall tissue of plants. A preliminary report on part of the work has already appeared (*21*). For a background to this work proximate and other analyses were also made.

EXPERIMENTAL WORK

Three kinds of plants were used to furnish material for analysis. Tomato (*Lycopersicum esculentum* Mill.) plants were grown in the greenhouse in the late fall and early winter of 1933 and in the field during the summers of 1934 and 1936. When the plants were 6 weeks old, a number of inoculations were made in the upper internodes with the crown gall organism, *Phytomonas tumefaciens*. A sufficient amount of gall tissue for analysis was produced in 6 weeks after inoculation. Simultaneously with the collection of the galls, sections of the stem immediately above and below the galls were taken to furnish samples of contiguous tissue. The samples from the greenhouse plants were placed in hot alcohol and stored until the following summer before being analyzed. Material from the plants grown in the field was analyzed immediately after harvesting.

A second source of material was the gall and normal tissue of sugar beets (*Beta vulgaris* L.) grown in the field and inoculated in the usual manner. A third source was the naturally occurring galls and contiguous bark from the roots of red raspberry (*Rubus strigosus* Michx.). The sugar-beet and raspberry samples were also analyzed immediately after harvesting. For this purpose 250-g samples were completely extracted with successive portions of hot 60-percent alcohol. The extracted residue was dried and ground to insure uniform sampling. It was employed for determinations of starch, pentosan, and uronic acid. The alcoholic extract was evaporated under reduced pressure to remove the alcohol, diluted with water to a definite volume, and then analyzed for sugar and for alpha amino, amide, ammonia, and nitrate nitrogen. Another portion of fresh tissue was dried at 100° C. for the determination of moisture, ash, ether extract, cellulose, and total nitrogen.

PROXIMATE AND OTHER ANALYSES

Analyses were made on the dried materials and solutions as follows: Ash, ether extract, total nitrogen, and pentosans by A. O. A. C. methods (*1*); ammonia, amide, and nitrate nitrogen by the method of Sessions and Shive (*27*); cellulose according to Kürschner and Hanak (*19*); starch by the method of Davis and Daish (*8*); uronic acid by the method of Dickson, Otterson, and Link (*10*); alpha amino nitrogen by the method of Van Slyke (*35*); and sugars according to Stiles, Peterson, and Fred (*30*). The results of the analyses are given in tables 1 and 2. Although considerable variability appears, there are certain conspicuous differences between crown gall, which grossly resembles embryonic tissue, and contiguous tissue. These differences are considered in the discussion.

TABLE 1.—*Chemical composition of normal stem and of gall tissues of tomato*

Analyses	Greenhouse material, 1933		Field material, 1934		Field [1] material, 1936	
	Stems	Galls	Stems	Galls	Stems	Galls
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Dry matter	10.4	10.3	14.0	11.4	16.8	10.1
Analyses on basis of dry matter:						
Ash	12.3	13.2	7.7	14.1	8.4	14.2
Ether extract	2.0	1.7	1.1	1.7	1.7	2.2
Total nitrogen	3.0	3.3	2.8	4.9	1.4	3.5
Alpha amino nitrogen	.1	.23	.40	.50	.25	.33
Amide nitrogen			.35	.26	.10	.08
Ammonia nitrogen			.15	.15	.06	.10
Nitrate nitrogen			.35	.24	.07	.12
Reducing sugars	4.5	1.9	4.6	2.3	3.9	5.1
Nonreducing sugars	3.0	2.3	.35	1.1	2.0	4.7
Starch	6.8	5.0	3.3	2.3	2.7	4.3
Cellulose	30.0	22.8	31.0	15.9	35.1	20.5
Pentosans	4.1	2.1	13.5	7.8	21.5	13.4
Uronic acids	8.9	10.9	10.4	9.1	3.7	13.4

[1] Produced during July and August 1936.

TABLE 2.—*Chemical composition of normal and of gall tissue of raspberry and sugar beets*

Analyses	Raspberry		Sugar beets [1]	
	Cortical tissue	Galls	Normal tissue	Galls
	Percent	*Percent*	*Percent*	*Percent*
Dry matter	40.7	17.9	20.0	16.1
Analyses on basis of dry matter:				
Ash	4.9	6.9	2.7	6.5
Ether extract	1.6	4.1	.4	1.0
Total nitrogen	1.4	3.1	.86	2.88
Alpha amino nitrogen	.33	.36	.2	.31
Amide nitrogen	.02	.05	.02	Trace
Ammonia nitrogen	.03	.23	.02	.02
Nitrate nitrogen	Trace	.0	.01	.03
Reducing sugars	5.7	8.0	.7	.8
Nonreducing sugars	1.9	.0	69.6	41.64
Starch	9.9	3.0	.1	.2
Cellulose	13.2	13.8	5.1	9.5
Pentosans	14.5	7.3	3.4	7.0
Uronic acids	10.1	8.2	4.5	4.1

[1] Produced during August and September 1937.

Glutathione determinations were made on galls, stems, and actively growing tips of tomato plants by the method of Okuda and Ogawa (*23*). This method gives both the oxidized and reduced forms of the sulphydryl group. The data (table 3) show that the tips of greenhouse plants contained much more glutathione than either the galls or stems. The galls from field plants had more than the stems.

Ascorbic acid determinations, also, were made on galls and on the tomato tissues by the method of Bessey and King (*5*). The results are given in table 4. As with glutathione, actively growing tips contained much more ascorbic acid than stems or galls in which relatively little was found.

TABLE 3.—*Glutathione content of crown gall and of contiguous tomato stems*

Material	Trials	Glutathione per 100 g of dry tissue		Material	Trials	Glutathione per 100 g of dry tissue	
		Reduced form	Oxidized form			Reduced form	Oxidized form
Grown in the greenhouse:	*Number*	*Milligrams*	*Milligrams*	Grown in the field:	*Number*	*Milligrams*	*Milligrams*
Galls	8	1.6	1.6	Galls	10	5.0	5.5
Stems	7	1.1	1.4	Stems	8	2.9	2.0
Active growing tips	4	7.5	3.7				

TABLE 4.—*Ascorbic acid content of crown gall and of contiguous tomato tissue*

Material	Trials	Ascorbic acid per 100 g of dry tissue	Material	Trials	Ascorbic acid per 100 g of dry tissue
Grown in the greenhouse:	*Number*	*Milligrams*	Grown in the field:	*Number*	*Milligrams*
Young galls	3	0.7	Galls	12	.1
Old galls	3	.8	Stems	12	.6
Young stems	3	.3			
Old stems	3	.6			
Active growing tips	3	2.2			

CATALASE

Catalase activity was determined by means of Appleman's apparatus as modified by Davis (*9*). The method involves the measurement in a gas burette of the oxygen liberated from hydrogen peroxide by the enzyme. Two-gram and 10-g samples of gall and contiguous stem tissue, respectively, were ground with a small amount of water, an excess of calcium carbonate, and a small amount of sand. After 2 minutes of grinding the macerated tissue was put through a fine wire gauze, thoroughly washed, and made up to 100 ml. Two milliliters of the gall extract and 10 ml of the stem extract were placed in flasks containing 10 ml of phosphate buffer (pH 7.0) and the flasks attached to a mechanical shaker in a 25° C. constant-temperature bath. When the contents of the flasks reached the desired temperature, 5 ml of 3-percent neutral hydrogen peroxide was added to the flasks, the shaker was started, and the volume of the evolved gas was measured in a gas burette at regular intervals. The readings were converted to standard pressures and temperatures. The galls liberated 160 percent more gas than the same weight of

contiguous stem tissue. The data in figure 1 are based on the average of 14 determinations on galls and an equal number of corresponding stems.

PEROXIDASE

Peroxidase activity was determined according to the method of Guthrie (*11*), based on the formation by the enzyme of phenol indophenol from *p*-phenylenediamine hydrochloride and alpha-naphthol, in the presence of hydrogen peroxide. One milliliter of a 10-percent plant extract prepared by grinding 10 g of tissue and diluting to 100 ml was used for each determination. As seen from figure 2, the galls were approximately 120 percent more active in the formation of indophenol than an equal weight of the contiguous stem. The data

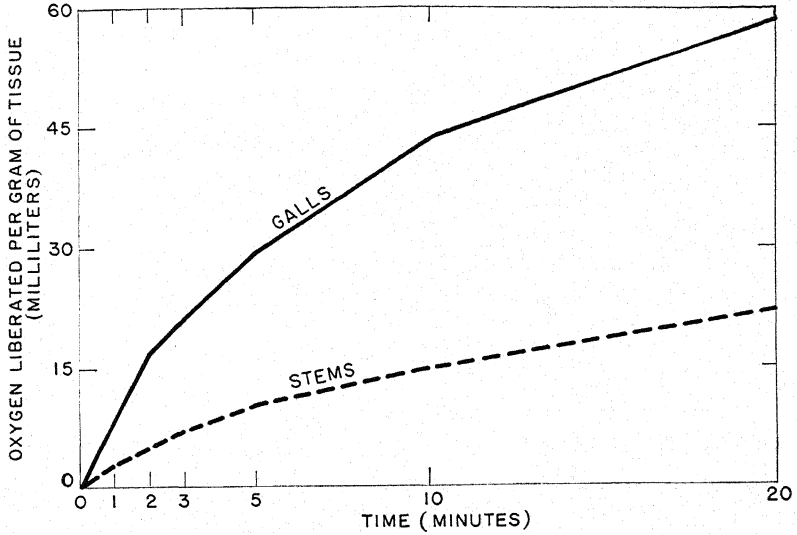


FIGURE 1.—Catalase activity of crown gall and of contiguous stem tissue of tomato.

are based on the average of 19 determinations on galls and an equal number of corresponding stems.

OXIDASE

Oxidase activity was measured by the Bunzel apparatus as modified by Harvey (*13*), based on the absorption of oxygen by pyrogallol. Twelve milliliters of a 1-percent solution of pyrogallol was placed in the larger of the two arms of the apparatus, and in the other, 5 ml of gall or stem juice together with 1 ml of phosphate buffer (pH 6.5). In the vial was placed 1 ml of concentrated sodium hydroxide. The apparatus was immersed in a 25° C. constant-temperature bath and agitated for 2 hours by means of a mechanical shaker. The amount of oxygen absorbed was measured by the difference in the mercury column. The galls showed approximately 130 percent more oxidase activity than the contiguous stem (fig. 3). The data are based on the average of nine determinations on galls and an equal number of corresponding stems.

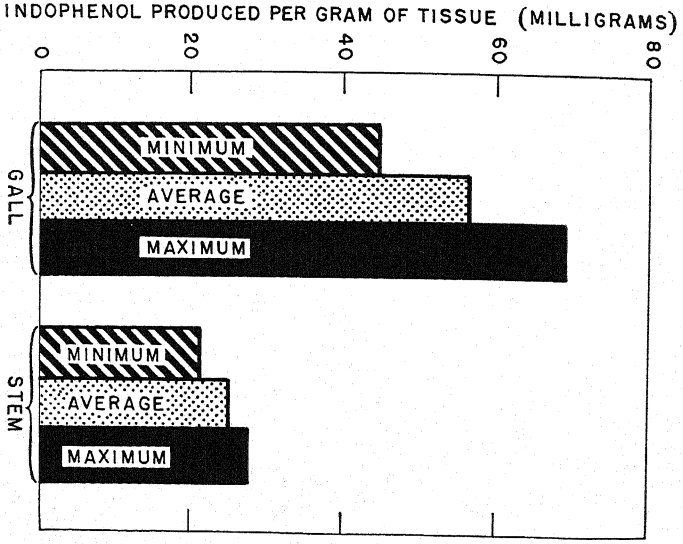


FIGURE 2.—Peroxidase activity of crown gall and of contiguous stem tissue of tomato.

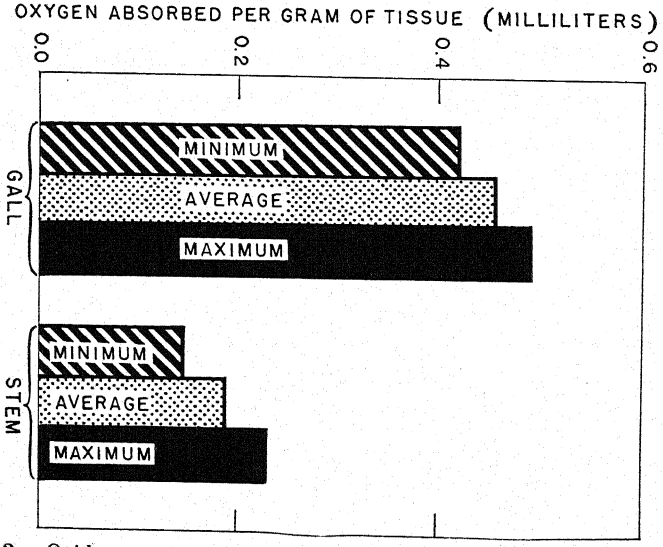


FIGURE 3.—Oxidase activity of crown gall and of contiguous stem tissue of tomato.

TYROSINASE

The activity of tyrosinase was determined by a modified method of Raper and Wormall (*24*), which involves the estimation of the unchanged tyrosine by means of phenol reagent at various intervals. Fifty milliliters of expressed crown gall juice were placed in a 500-ml flask containing 200 ml of 0.05-percent tyrosine solution buffered with borate to pH 8.0. The flasks were immersed in a 25° C. constant-temperature bath and rapidly aerated. At regular intervals 10-ml samples were withdrawn and pipetted into 0.5 ml of 10-percent acetic acid and the mixture was brought to boiling. The solution was filtered into 100-ml volumetric flasks and the precipitate washed well with hot water. The filtrate was diluted to about 50 ml; 5 ml

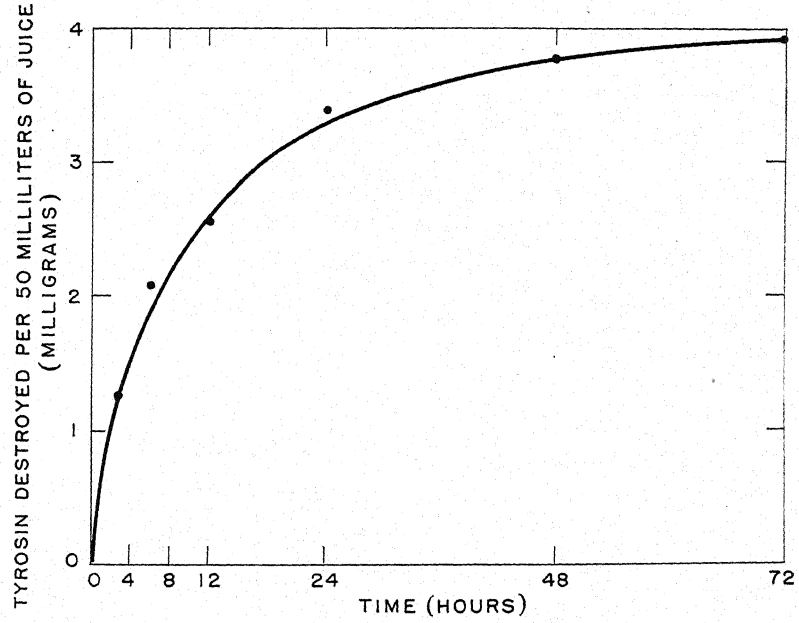


FIGURE 4.—The rate of destruction of tyrosine by the tyrosinase of crown gall from tomato.

of phenol reagent and 25 ml of saturated sodium carbonate were added. After standing for one-half hour the flasks were made up to 100 ml and compared colorimetrically with a standard containing 1 to 4 mg of tyrosine in 100 ml prepared at the same time as the unknown. In 8 hours 50 ml of crown gall juice destroyed one-half of the tyrosine, whereas no loss was detected from a similar preparation of stem tissue (fig. 4). The data are based on the average of six determinations on galls and an equal number of corresponding stems.

HYDROGEN-ION CONCENTRATION

Hydrogen-ion concentration of galls and of contiguous stem both above and below the galls was determined with a glass electrode. About 3 g of tissue was mascerated in 10 ml of water, and the determinations on the liquid were made within 3 minutes. Nineteen

representative determinations gave the following averages: Gall tissue, pH 5.72; stem tissue pH 5.79. This indicates that no significant difference existed between the tissues, when large masses were thus examined. It is possible, however, that significant differences exist between different portions of a given tissue, as suggested by Berridge (*2*).

DISCUSSION

The analyses made of the galls and contiguous tissue indicate a condition in the galls similar to that found in young plant tissues. The ash, total nitrogen, and simple forms of nitrogen in tomato are generally higher, whereas nonmetabolically active materials, such as cellulose and pentosans, are lower in the galls than the host plant. The cellulose and pentosans of sugar beets are higher in galls than in contiguous uninfected tissue. The difference between the two tissues in all three host plants is particularly noteworthy with respect to the more highly organized nitrogen. This conclusion is apparent if the sum of the nitrogen fractions is subtracted from the total. The difference in nitrogen thus obtained, probably polypeptide or protein in character, is from two to four times as high in the gall as in normal tissue. So far as the analyses reported are concerned the papers cited in the introduction are in general confirmed by the present studies.

Glutathione is found in greater abundance in the galls than in contiguous stems, but it is even more abundant in the actively growing tips. This situation is in accordance with Hammett's views on the role of glutathione in the organization of protein.

The activity of the oxidizing enzymes may be considered a measure of the metabolic status of the tissue, and hence may serve as an indicator of the physiological response of plants to various treatments. Since the galls are in a highly active vegetative state, it is not surprising to find the concentration of catalase, peroxidase, and oxidase greater in the galls than in the stems. It is doubtful that the increase in catalase is owing to the hydrogen-ion concentration as suggested by Harvey (*13*), since no significant difference was found in the pH value of the galls and neighboring tissue used. Bristol (*6*) and Lantz (*20*) reported an inhibitory action of catalase on the oxidation processes within the cell. Unpublished determinations in the writers' laboratories on the rate of respiration, as measured by the Barcroft apparatus, indicated a great increase in the uptake of oxygen in the galls over that of the contiguous uninoculated tissue. It appears that under these conditions an environment would be created in which there would be an insufficient amount of oxygen for some of the cells. This, according to Smith (*28*), and Smith, Brown, and Townsend (*29*), would compel these cells to divide if they were to live.

The tyrosinase activity of the gall tissue deserves special comment. Although traces of this enzyme were observed both in a culture of *Phytomonas tumefaciens* and in the tomato plant, the tyrosinase of the galls is so great that the amounts cannot be quantitatively compared. Alpha amino nitrogen and tyrosinase activity were, respectively, 20 and 200 percent greater in the galls than in the contiguous stem. Tottingham, Nagy, and Ross (*33*) found a condition similar to this in abnormal potatoes. Rapidity of oxidation, indicated by

the increase in oxidative enzymes, and presence of a large amount of amino acids may favor both production and activity of tyrosinase in the galls. Nobutani (*22*) found a marked stimulation of tyrosinase activity on a *p*-cresol substrate by a number of amino acids.

All of the enzyme determinations are calculated on the basis of whole tissue and, therefore, may not be on a comparable basis. The contiguous tissue contains larger proportions of inert material, such as cellulose and pentosans, than is found in the galls. Since the metabolically active protoplasm is largely composed of nitrogenous material with only a small amount of carbohydrates, the nitrogen content instead of whole tissue may be used as another basis for calculating enzyme activity. But even on the basis of total nitrogen the galls were higher in catalase, oxidase, and peroxidase, by 86, 73, and 57 percent, respectively.[4]

SUMMARY

A number of analyses have been made on the galls and contiguous tissue from tomatoes, raspberry, and sugar beets. In general the composition of gall tissue resembled that of young plants, being high in nitrogen and low in fibrous material. In sugar beets, however, the galls were more fibrous than the succulent host plant. The composition of the galls and contiguous tissues varied greatly depending upon the time of harvest and the species of plant.

The glutathione content of the tomato galls was greater than that of the contiguous stems, but was much lower than that of the growing tips.

The more metabolically active tissue produced the greater amount of ascorbic acid.

The hydrogen-ion concentration of stems and galls of tomato tissue were approximately the same.

Catalase, oxidase, and peroxidase activity, on the wet-weight basis, were 160, 130, and 120 percent greater, respectively, in the tomato galls than in the contiguous tomato stem tissues. Calculated on the basis of total nitrogen instead of wet weight, the figures for galls were 86, 73, and 57 percent greater, respectively, than those for stems.

Extracts from tomato galls rapidly destroyed tyrosine but a similar preparation from stems showed no tyrosinase activity.

LITERATURE CITED

(1) Association of Official Agricultural Chemists.
1930. official and tentative methods of analysis . . . Ed. 3, 593 pp., illus. Washington, D. C.

(2) Berridge, Emily M.
1930. studies in bacteriosis. xvii. acidic relations between the crown-gall organism and its host. Ann. Appl. Biol. 17: 280–283, illus.

(3) Berthelot, Albert, and Amoureux, G.
1936. recherches sur la composition chimique des tumeurs de la betterave déterminées par bacillus tumefaciens. Compt. Rend. Soc. Biol. [Paris] 123: 942–944.

(4) ——— and Amoureux, G.
1936. sur la teneur en glutathion et en acide ascorbique des tumeurs de la betterrave déterminées par bacillus tumefaciens. Compt. Rend. Soc. Biol. [Paris] 123: 944–946.

[4] In making these calculations the 1934 data were used as all enzyme analyses were made on that year's samples.

(5) BESSEY, OTTO A., and KING, C. G.
1933. THE DISTRIBUTION OF VITAMIN C IN PLANT AND ANIMAL TISSUES, AND ITS DETERMINATION. Jour. Biol. Chem. 103: 687–698.
(6) BINET, LÉON, and MAGROU, J.
1931. GLUTATHION, CROISSANCE ET CANCER DES PLANTES. Compt. Rend. Acad. Sci. [Paris] 192: 1415–1416.
(7) BRISTOL, LEVERETT DALE.
1916. AN ENZYME THEORY OF CANCER ETIOLOGY. Med. Rec. 89: 180–191.
(8) DAVIS, WILLIAM A., and DAISH, ARTHUR JOHN.
1914. METHODS OF ESTIMATING CARBOHYDRATES. II. THE ESTIMATION OF STARCH IN PLANT MATERIAL. THE USE OF TAKA-DIASTASE. Jour. Agr. Sci. [England] 6: [152]–168, illus.
(9) DAVIS, WILMER E.
1926. THE USE OF CATALASE AS A MEANS OF DETERMINING THE VIABILITY OF SEEDS. Assoc. Off. Seed Analysts Proc. (1925) 18: 33–39, illus.
(10) DICKSON, ALLAN, D., OTTERSON, HENRY, and LINK, KARL PAUL.
1930. A METHOD FOR THE DETERMINATION OF URONIC ACIDS. Jour. Amer. Chem. Soc. 52: 775–779, illus.
(11) GUTHRIE, JOHN D.
1931. A METHOD FOR THE DETERMINATION OF PEROXIDASE ACTIVITY. Jour. Amer. Chem. Soc. 53: 242–244.
(12) HAMMETT, FREDERICK S.
1934. A POSSIBLE EXPLANATION OF THE FUNCTION OF GLUTATHIONE IN DEVELOPMENTAL GROWTH. Science 79: 457.
(13) HARVEY, R. B.
1920. APPARATUS FOR MEASUREMENT OF OXIDASE AND CATALASE ACTIVITY. Jour. Gen. Physiol. 2: 253–254, illus.
(14) HAUSEN, S. v.
1936. THE ROLE OF VITAMIN C IN THE GROWTH OF HIGHER PLANTS. Ann. Acad. Sci. Fennicae (A) 46: 1–134, illus. [Also in Biochem. Ztschr. 288: 378–392.]
(15) KLEIN, G., and KEYSSNER, E.
1932. BEITRÄGE ZUM CHEMISMUS PFLANZLICHER TUMOREN. I. MITTEILUNG: STICKSTOFFBILANZ. Biochem. Ztschr. 254: [251]–255.
(16) ——— and ZIESE, W.
1932. BEITRÄGE ZUM CHEMISMUS PFLANZLICHER TUMOREN. III. MITTEILUNG: DER KATALASEGEHALT VON PFLANZLICHEN TUMOREN IM VERGLEICH ZUM KATALASEGEHALT GESUNDEN PFLANZENGEWEBES. Biochem. Ztschr. 254: 264–285, illus.
(17) ——— and ZIESE, W.
1933. BEITRÄGE ZUM CHEMISMUS PFLANZLICHER TUMOREN. IV. MITTEILUNG: ÜBER PEROXYDASE IN PFLANZLICHEN TUMOREN. Biochem. Ztschr. 267: [22]–25.
(18) KLUYVER, A. J.
1932. MICROBIAL METABOLISM AND ITS BEARING ON THE CANCER PROBLEM. Science 76: 527–532.
(19) KÜRSCHNER, K., and HANAK, A.
1930. ZUR BESTIMMUNG DER SOG. ROHFASER. EIN NEUES VERFAHREN DER BESTIMMUNG DER ROHCELLULOSE IN KAKAO. Ztschr. Untersuch. Lebensmtl. 59: 484–494, illus.
(20) LANTZ, C. W.
1927. RESPIRATION IN CORN WITH SPECIAL REFERENCE TO CATALASE. Amer. Jour. Bot. 14: 85–105, illus.
(21) NAGY, R., PETERSON, W. H., and RIKER, A. J.
1937. COMPARISON OF ENZYMES IN CROWN-GALL AND NONINOCULATED PLANT TISSUE. (Abstract) Phytopathology 27: 136.
(22) NOBUTANI, FUJIO.
1936. STUDIES ON TYROSINASE. II. THE ACTION OF THE POTATO TYROSINASE ON VARIOUS PHENOLIC SUBSTANCES AND THE EFFECT OF ADDITION OF VARIOUS AMINO COMPOUNDS ON THAT TYROSINASE SYSTEM. Jour. Biochem. 23: 455–485, illus.
(23) OKUDA, YUZURU and OGAWA, MASAYOSHI.
1933. ON THE METHOD OF QUANTITATIVE ESTIMATION OF GLUTATHIONE IN TISSUES. Jour. Biochem. 18: 75–86.

(24) RAPER, HENRY STANLEY, and WORMALL, ARTHUR.
1923. THE TYROSINASE-TYROSINE REACTION. Biochem. Jour. 17: [454]–469, illus.
(25) ROBINSON, WILFRID.
1927. SOME FEATURES OF CROWN GALL IN PLANTS IN REFERENCE TO COMPARISONS WITH CANCER. Roy. Soc. Med., Sect. Trop. Diseases Parasitol, Proc. 20: 53–56. [Whole pag. [1507]–1510.]
(26) SAGEN, H. E., RIKER, A. J., and BALDWIN, I. L.
1934. STUDIES ON CERTAIN PHYSIOLOGICAL CHARACTERS OF PHYTOMONAS TUMEFACIENS, PHYTOMONAS RHIZOGENES, AND BACILLUS RADIOBACTER. PART I. Jour. Bact. 28: 571–595, illus.
(27) SESSIONS, A. C., and SHIVE, J. W.
1928. A METHOD FOR THE DETERMINATION OF INORGANIC NITROGEN IN PLANT EXTRACTS. Plant Physiol. 3: 499–511, illus.
(28) SMITH, ERWIN F.
1920. PRODUCTION OF TUMORS IN THE ABSENCE OF PARASITES. Arch. Dermatol. and Syphilology 2: [176]–180.
(29) ——— BROWN, NELLIE A., and TOWNSEND, C. O.
1911. CROWN-GALL OF PLANTS: ITS CAUSE AND REMEDY. U. S. Bur. Plant Indus. Bull. 213, 215 pp., illus.
(30) STILES, H. R., PETERSON, W. H., and FRED, E. B.
1926. A RAPID METHOD FOR THE DETERMINATION OF SUGAR IN BACTERIAL CULTURES. Jour. Bact. 12: 427–439.
(31) STROHMER, F., and STIFT, A.
1892. CHEMISCHES ÜBER DEN WURZELKROPF. Österr.-Ungar. Ztschr. Zuckerindus. u. Landw. 21: 262–264.
(32) SYLWESTER, ERHARDT P., and COUNTRYMAN, MARY C.
1933. A COMPARATIVE HISTOLOGICAL STUDY OF CROWNGALL AND WOUND CALLUS ON APPLE. Amer. Jour. Bot. 20: 328–340, illus.
(33) TOTTINGHAM, W. E., NAGY, RUDOLPH, and ROSS, A. FRANK.
1936. THE PROBLEM OF CAUSES OF BLACKENING IN COOKED POTATOES. Amer. Potato Jour. 13: [297]–309, illus.
(34) TOWNSEND, C. O.
1915. FIELD STUDIES OF THE CROWN-GALL OF SUGAR BEETS. U. S. Dept. Agr. Bull. 203, 8 pp., illus.
(35) VAN SLYKE, DONALD D.
1913. THE GASOMETRIC DETERMINATION OF ALIPHATIC AMINO NITROGEN IN MINUTE QUANTITIES. Jour. Biol. Chem. 16: 121–124.
(36) VIRTANEN, ARTTURI I.
1936. VITAMINS AND PLANTS. Nature [London] 137: 779–780.

U. S. GOVERNMENT PRINTING OFFICE: 1938

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 57 WASHINGTON, D. C., OCTOBER 1, 1938 No. 8

STUDIES ON PROBABLE DAMAGE BY BLISTER RUST IN SOME REPRESENTATIVE STANDS OF YOUNG WESTERN WHITE PINE [1]

By THOMAS W. CHILDS and J. W. KIMMEY, *assistant pathologists*,[2] *Division of Forest Pathology, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

It has long been known that white-pine blister rust (*Cronartium ribicola* Fisch.) is able to kill pines of susceptible species. Quantitative studies of damage by this disease are obviously essential to the formulation of satisfactory management and disease-control policies for white pine stands. Snell (*7, 8, 9, 10*) [3] and others have discussed the effect of the rust on young eastern white pines (*Pinus strobus* L.). Lachmund (*5*) has described examples of damage to commercially immature western white pines (*P. monticola* Dougl.), and Buchanan (*1*) has recently demonstrated the destructiveness of the disease to merchantable trees of the same species.

This paper is concerned with the relationship between intensity of infection and degree of damage, and with some of the more important factors affecting this relationship, in stands of western white pine less than 50 feet in height. Even in very small trees the killing of occasional branches is of little or no material importance. When conditions for pine infection are very favorable, cankers may sometimes be so numerous as to cause the death of trees simply by killing most of the branches individually (i. e., without girdling the trunks) (*5*), but in such cases the amount of infection present far exceeds the minimum necessary to cause the same damage by trunk girdling within a few additional years. Throughout this paper, therefore, only those cankers that have entered or may be expected to enter the trunks are considered to be injurious to the host.

METHODS

The studies were generally confined to areas where practically all of the cankers had originated in a single year or in two consecutive years as indicated by Lachmund's method (*3*) of determining the age of infection on western white pine, and data were usually taken only on those cankers that appeared to have originated during the year or years of heaviest infection. In most cases the trees were examined as soon as the cankers were large enough to be found easily (about 5 years after the occurrence of the infection). The following data were

[1] Received for publication May 31, 1938; issued November 1938.

[2] Stationed at the U. S. Forest Pathology Field Laboratory, Portland, Oreg., maintained in cooperation with the United States Forest Service. The writers wish to acknowledge their indebtedness to T. S. Buchanan and J. L. Mielke, of the Division of Forest Pathology; to H. G. Lachmund, C. J. Nusbaum, and C. N. Partington, formerly of that Division; and to the late A. T. Davidson, in charge of blister rust investigations for the Dominion Government, to H. T. Güssow, Dominion botanist, and to other members of the Dominion and Provincial Governments of Canada for their friendly cooperation during the course of the investigations in that country.

[3] Italic numbers in parentheses refer to Literature Cited, p. 568.

recorded for every infected tree: Height at time of infection and at time of examination, length and width of crown at time of infection (except at Brackendale, where crown dimensions were taken as of the year of examination), number of cankers, and number of cankers capable of causing damage. In British Columbia the following additional data were usually taken on each canker: Distance from ground, distance from trunk, and probable date when damage would be caused. Where not otherwise specified, all data presented are as of the year in which infection occurred.

Lachmund (*4, pp. 491–499*) has published a detailed description of the principal factors that determine whether or not a given canker will ever enter the trunk, and of the method of calculating the time and severity of the injury that will be caused by cankers able to enter. The studies reported in this paper are based almost entirely upon such calculations, since few stands of western white pine have as yet been exposed to the rust long enough for damage from even the older cankers to manifest itself fully, and since successive waves of infection are usually so heavy as to prevent any determination of the damage that might result from smaller numbers of cankers. For the same reasons it has been impossible to make extensive comparisons of calculated results with actual results; however, a careful reexamination (about 5 years after the original examination) of 959 cankers on one of the study areas (Revelstoke) indicated that damage, although somewhat slower than had been expected, would conform very closely to the original calculations.

The potential effect of each individual canker was determined without reference to any other canker. For example, a canker that would kill its host in 20 years if it were the only canker present was recorded as a fatal infection even if another canker was present that would kill in 15 years. Cankers were classified in three groups: (1) Those that would kill (this group included a majority of the cankers capable of damaging, even in trees from 40 to 50 feet tall), (2) those that would seriously injure (*4, p. 498*), and (3) those that would never enter the trunk. It makes little difference from a practical standpoint, however, whether a tree is killed or only injured to such an extent that its potential value is largely destroyed; accordingly, throughout this paper no distinction is made between cankers capable of killing and those capable only of causing serious injury.

STUDY AREAS

The studies were initiated in 1928 on an area of level land at a low elevation near Brackendale in the coastal region of British Columbia. Very few ribes were present on this study area, but large numbers of some of the most susceptible species, including *Ribes bracteosum* Dougl., *R. divaricatum* Dougl., and a few plantings of *R. nigrum* L., occurred around it. Western white pines ranging from 10 to 60 feet in height were mixed with Douglas fir (*Pseudotsuga taxifolia* (Lam.) Britt.) and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) in a stand of rather uneven density. Blister rust became established in this locality about 1913. Moderately heavy pine infection occurred during 1917 and 1918, and again during 1920 and 1921. Only those cankers originating during 1917 and 1918 were included in the study. Since practically all of the sporidia came from ribes at some distance from the study area, pine infection was distributed fairly uniformly throughout the stand.

The studies were continued during 1929 to 1932, inclusive, near Revelstoke,[4] in eastern British Columbia about 135 miles north of the international boundary. This area was situated on a steep slope just above the Columbia River, and ranged from about 1,800 to about 2,400 feet in elevation. *Ribes lacustre* (Pers.) Poir. occurred commonly along a small stream, and scattered bushes of the same species were present in various other parts of the area. The nearest ribes of any other species were several miles away. Western white pines, mostly less than 25 feet tall, were mixed with other conifers, willows, aspen, alders, etc., in a stand of rather irregular density. The rust probably became established here about 1917. A light wave of pine infection occurred in 1922, a few additional cankers originated in 1924 and 1925, and several thousand cankers originated during 1927 and 1928. Data were taken on all cankers, regardless of their age. The cankers on this area have been separated into two groups: Those originating in 1922, and those originating during 1924 to 1928, inclusive. All cankers of the latter group are herein assumed, for the sake of convenience, to have originated in 1927 (the year in which more than 80 percent of the total infection occurred).

An area[5] at an elevation of approximately 2,000 feet near Hunter's Siding (about 50 miles south of Revelstoke) was examined during 1930 to 1933, inclusive. Western white pines, averaging 443 per acre, were mixed with other conifers and various hardwoods in a fairly dense stand. Infection here originated in 1928 and in 1930 from inoculated ribes at the center of the area. Data were taken on all cankers. Tree dimensions are herein given as of 1928 (the year in which slightly more than 50 percent of the infection occurred).

By 1933 the disease had become common and conspicuous on pines at several infection centers in Idaho. The following five areas in that State were examined during that year: Crystal Creek (east of Fernwood, Benewah County), St. Maries River (east of Clarkia, Shoshone County), Gold Center Creek (east of Clarkia), Deep Creek (south of Elk River, Clearwater County), and Elk River (south of Elk River). These areas were situated on gentle to moderate slopes at elevations ranging from about 2,000 to about 3,500 feet. White pine was the principal tree species on all the areas except the one at Deep Creek, where Douglas fir and western hemlock were also abundant. The stands were moderately dense at Crystal Creek and St. Maries River, somewhat less so at Deep Creek, irregular but generally open at Elk River, and quite open at Gold Center Creek. In every case, highly susceptible stream-type ribes had been abundant in the immediate vicinity of the pines, but these bushes had recently been destroyed except at Crystal Creek. Practically all of the cankers, and all of those included in the study, were of 1927 origin at Crystal Creek and St. Maries River, of 1928 origin at Deep Creek and Elk River, and of 1930 origin at Gold Center Creek.

On the Revelstoke, Hunter's Siding, and Gold Center Creek areas, detailed data were taken on all trees, both infected and uninfected. On the rest of the Idaho areas, and at Brackendale, no record was kept of uninfected trees, and infected trees were ignored whenever a

[4] A more detailed description of this area is given by Mielke (*6*). The tree and canker bases used in this paper differ slightly from those used by Mielke, since insufficient data on some of the cankers necessitated their omission and since a few cankers that may have originated in 1929 have been included.

[5] Described in more detail by Buchanan and Kimmey (*2*).

TABLE 1.—*Number of cankers and infected trees, by tree-height classes, used on the various study areas*

Tree-height class (feet)	Brackendale		Revelstoke [1]		Revelstoke [2]		Hunter's Siding		Crystal Creek		St. Maries River		Gold Center Creek		Deep Creek		Elk River		Total	
	Infected trees	Cankers	Infected trees	Cankers	Infected trees	Cankers	Infected trees	Cankers	Infected trees	Cankers	Infected trees	Cankers	Infected trees	Cankers	Infected trees	Cankers	Infected trees	Cankers	Infected trees	Cankers
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
0.1–5.0	0	-------	21	21	206	239	11	20	30	118	30	171	119	178	68	83	31	51	516	881
5.1–10.0	13	39	61	64	469	739	30	42	32	370	32	189	69	189	73	118	52	169	831	1,919
10.1–15.0	51	242	93	98	509	1,025	41	85	31	216	30	175	8	19	25	59	45	210	833	2,120
15.1–20.0	51	177	113	124	598	1,628	21	60	30	191	29	268	0	-------	8	23	38	312	888	2,783
20.1–25.0	50	315	18	18	786	2,603	14	19	33	336	31	305	0	-------	2	29	33	268	967	3,893
25.1–30.0	51	377	0	-------	546	2,215	2	2	30	252	31	277	0	-------	0	-------	32	410	692	3,533
30.1–35.0	50	433	0	-------	201	1,098	0	-------	30	192	30	354	0	-------	1	11	31	402	343	2,490
35.1–40.0	50	467	0	-------	33	213	0	-------	30	329	30	229	0	-------	0	-------	37	369	180	1,607
40.1–45.0	28	255	0	-------	3	15	0	-------	31	332	31	322	0	-------	0	-------	25	173	118	1,097
45.1–50.0	10	112	0	-------	0	-------	0	-------	30	513	27	201	0	-------	0	-------	19	154	86	980
Total	354	2,417	306	325	3,351	9,775	119	228	307	2,849	301	2,491	196	386	177	314	343	2,518	5,454	21,303

[1] Infection originating in 1922.

[2] Infection originating in 1924 to 1928, inclusive.

sufficient representation of the height class to which they belonged had already been secured on that area.

All of the areas, in both British Columbia and Idaho, were representative of good white pine sites in their respective regions. Practically all of the trees included in these studies were dominant or codominant. The number of cankers and the number of infected trees on each of the study areas are given by tree-height classes in table 1.

RELATIONSHIP BETWEEN INFECTION AND DAMAGE IN INDIVIDUAL TREES

Infections occasionally occur on the needle-bearing portion of the main stem and on the small secondary branches common in the interior of western white pine crowns, but most of the foliage, and hence most of the infections, occur near the ends of the primary branches and their principal ramifications. In small trees (i. e., up to about 5 feet in height) the branches are so short that a majority of the cankers are able to grow into the main stem, where their action is almost invariably fatal because of the extensive development of the crown above the infected whorl during the period between infection and stem girdling, and because cankers on stems of young trees are usually able to continue downward growth until the base of the crown is reached. In larger trees, cankers may be grouped in three intergrading classes, in decreasing order of frequency, as follows: (1) Those originating in the lower part of the crown, which are generally unable to enter the trunk because they are usually situated near the ends of long branches; (2) those originating in the intermediate part of the crown, which are frequently able to enter the trunk and in such cases are almost always fatal; (3) those originating in the upper part of the crown, which are usually able to enter the trunk but which may merely injure rather than kill if a large part of the crown still exists below the infected whorl when the trunk is girdled and if the canker is subsequently unable to grow down to the base of the crown.

Most cankers occur in the lower portions of the crowns, principally because much greater quantities of foliage are present there than in the tops of the trees. It might therefore be expected that the percentage of cankers capable of causing damage would be much less in larger trees, whose lower branches are too long to be traversed by very many of the cankers originating near their tips, than in smaller trees. Figures 1 and 2 show this to be the case. Many of the differences apparent between the various curves in these two figures were also at least partially attributable to differences in crown width. For example, crown widths (by 5-foot tree-height classes) for trees from 15.1 to 50.0 feet tall ranged from 6 to 10 feet at Crystal Creek and from 8 to 16 feet at St. Maries River, while percentages of cankers capable of causing damage in these height classes (fig. 2) ranged from 21 to 32 and 15 to 23, respectively. These areas appeared fairly similar in other respects, and it seems highly probable that crown width was the factor principally responsible for the difference between these two percentage ranges. On the other hand, the percentage of cankers capable of causing damage to trees of any given height class on the Revelstoke area (fig. 1) was distinctly greater in the infection wave of 1922 than in that of 1927, although average crown widths by height classes in the latter year did not differ appreciably from those in the former. In

1922 this stand was relatively open, and in only a few cases was suppression of infected branches likely to prevent growth of cankers into the trunks. By 1927 the coniferous and hardwood cover was begin-

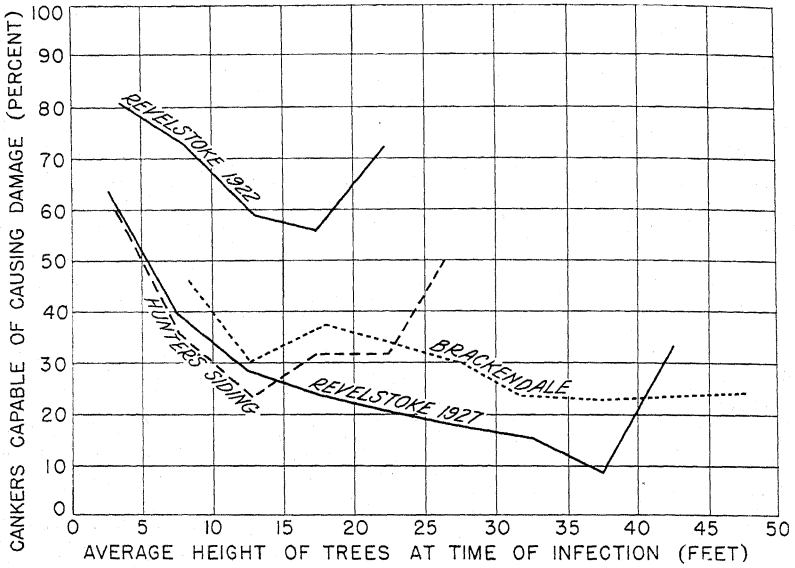


FIGURE 1.—Percentage of cankers capable of causing damage in the various height classes on study areas in British Columbia.

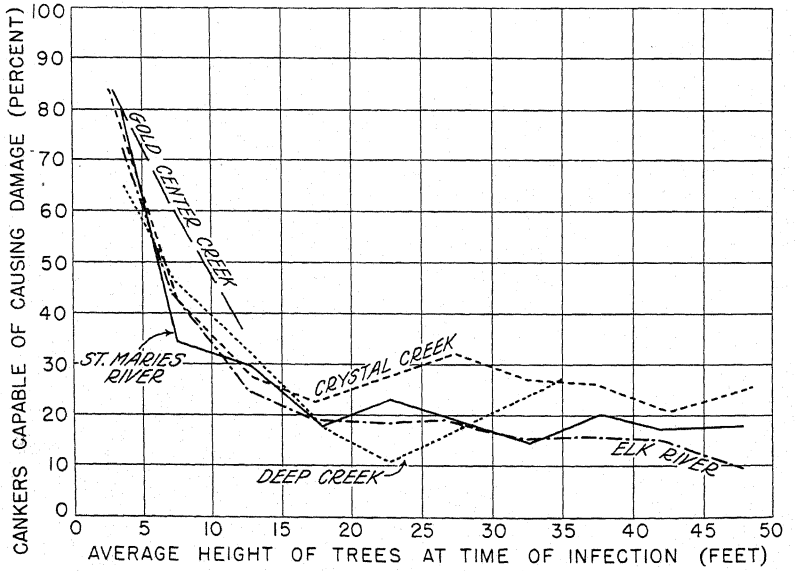


FIGURE 2.—Percentage of cankers capable of causing damage in the various height classes on study areas in Idaho.

ning to close over much of the area, and it was obvious that cankers of 1927 origin at increasing distances from the trunks were progressively less likely to enter than were those of 1922 origin (table 2).

TABLE 2.—*Percentages of cankers, originating at different distances from the trunks on the Revelstoke area, capable of causing damage* [1]

Distance of canker origin from trunk (feet)	Canker basis [2]		Cankers capable of causing damage		Distance of canker origin from trunk (feet)	Canker basis [2]		Cankers capable of causing damage	
	1922 infection	1927 infection	1922 infection	1927 infection		1922 infection	1927 infection	1922 infection	1927 infection
	Number	*Number*	*Percent*	*Percent*		*Number*	*Number*	*Percent*	*Percent*
0.1–1.0	59	536	93	87	5.1–6.0	6	468	0	[3] +
1.1–2.0	116	1,418	81	49	6.1–7.0	0	121	----	2
2.1–3.0	85	1,786	44	16	7.1–8.0	0	28	----	0
3.1–4.0	37	1,448	22	3	8.1–9.0	0	7	----	0
4.1–5.0	14	1,054	7	[3] +					

[1] Data from all tree-height classes.
[2] Total basis differs from that given in table 1 because data on distance from trunk were not available for all cankers.
[3] The plus signs signify percentages greater than 0 but less than 0.5.

Unfortunately, the available data are insufficient to illustrate the relation between crown length and severity of damage. Other things being equal, it is evident that shortening of the crowns will not result in a proportional reduction in the number of cankers capable of damaging, since such cankers usually originate in the upper portions of the crowns. In reality, however, trees subjected to lateral suppression differ in many respects from those receiving full side light, and the probable extent of damage from a given degree of infection in a closed stand consequently cannot be even approximately predicted from studies of more open stands, or vice versa.

Table 3 shows that the period between infection and actual occurrence of damage is longer, on an average, in large than in small trees. The most rapid increase in the length of this period apparently takes place in trees less than about 10 feet tall. Both crown widths and distances of cankers in general from the trunks are considerably greater in larger trees, but cankers near the ends of long branches are rarely able to cause damage and the average distance of potentially damaging cankers from the trunks of such trees is accordingly only slightly greater than in trees 10 feet tall. Then, too, cankers grow more rapidly on thick branches than on slender ones (*4*), and on long branches may consequently grow at rates that partially compensate for the greater distances to be traversed.

Lachmund (*4*) found canker growth rates to be somewhat higher on branches of the same diameter at low elevations in the coastal region of British Columbia than at Revelstoke. The average time required for damage to occur in a given height class might therefore be expected to be somewhat shorter at Brackendale than at Revelstoke, since table 3 shows that average distances from trunks to points of origin of potentially damaging cankers were approximately the same on these two areas. At Brankendale, however, the branches were unusually slender and growth of cankers was correspondingly slow.

From the foregoing discussion it will be seen that numerous factors may cause appreciable variation in the relationship between intensity of infection and probability of damage by blister rust, even in adjacent trees. The data presented herein indicate, however, that consider-

able damage can be caused by relatively light infection in trees less than 50 feet tall, and that such damage will generally occur before commercial maturity under western standards is attained.

TABLE 3.—*Distance from trunk to point of origin of potentially damaging cankers, and average time required for damage to occur*

Tree height class (feet)	Revelstoke [1]		Brackendale		Tree height class (feet)	Revelstoke [1]		Brackendale	
	Average distance from trunk to origin of potentially damaging cankers	Average time from infection to damage	Average distance from trunk to origin of potentially damaging cankers	Average time from infection to damage		Average distance from trunk to origin of potentially damaging cankers	Average time from infection to damage	Average distance from trunk to origin of potentially damaging cankers	Average time from infection to damage
	Feet	*Years*	*Feet*	*Years*		*Feet*	*Years*	*Feet*	*Years*
0.1–5.0	0.5	11			25.1–30.0			2.0	20
5.1–10.0	1.2	16	1.1	17	30.1–35.0			1.9	20
10.1–15.0	1.7	18	1.8	18	35.1–40.0			2.1	21
15.1–20.0	2.0	20	1.6	19	40.1–45.0			2.4	23
20.1–25.0	2.1	20	1.9	19	45.1–50.0			2.1	22

[1] Infection originating in 1922.

The effect of blister rust on trees more than about 50 feet in height cannot be even approximately predicted by extrapolation of the data herein presented. Crowns change in form and character as the rate of height growth diminishes, and these changes become most pronounced in trees between 50 and 100 feet tall. Since the structure of the crowns very largely determines the extent of the damage likely to result from a given number of cankers, it is evident that conclusions as to the probable liability of infected mature and nearly mature trees to damage can be derived only from studies of such trees.

EFFECT OF TREE SIZE ON LIABILITY TO INFECTION AND RESULTANT DAMAGE

Large trees usually have much more foliage and, as a result, are much more subject to infection than are smaller trees exposed to the same conditions. Consequently, although the percentage of cankers capable of causing damage decreases with increasing tree size (figs. 1 and 2), percentages of total trees damaged are frequently, if not always, greater in the larger size classes (up to at least 40 feet in height) of uneven-aged stands of reproduction. This is shown in table 4, where infection and damage percentages in the various height classes are given for the Revelstoke, Hunter's Siding, and Gold Center Creek areas.

This relationship between size of tree and liability to damage appears fundamentally unlike that described by Snell in *Pinus strobus* (*7*, *8*). Snell's results cannot be directly compared with those of the present study, because he considered as damaged only those trees that would be killed by the rust at ages of less than 50 years, and because his estimates of damage in the various height classes are expressed as percentages of infected trees instead of as percentages of

total trees; his data and discussion, however, indicate that infection of *P. strobus* is most severe among the smallest trees. The dissimilarity between the two species must therefore be partially attributable to differences in conditions governing distribution of infection, and possibly also to differences in development of and host reaction to established cankers, as well as to the use of different criteria in the estimation of damage.

TABLE 4.—*Infection and damage, by tree-height classes, on the Revelstoke,*[1] *Hunter's Siding, and Gold Center Creek areas*

Area and tree-height class (feet)	Basis		Average per tree		Trees infected	Trees damaged
	Trees	Cankers	Total cankers	Cankers capable of damaging		
Revelstoke:	*Number*	*Number*	*Number*	*Number*	*Percent*	*Percent*
0.1–5.0	3,107	239	0.1	[2] +	7	5
5.1–10.0	2,046	739	.4	0.1	23	12
10.1–15.0	1,218	1,025	.8	.2	42	19
15.1–20.0	1,160	1,628	1.4	.3	52	23
20.1–25.0	1,258	2,603	2.1	.4	62	27
25.1–30.0	812	2,215	2.7	.5	67	29
30.1–35.0	274	1,098	4.0	.6	73	36
35.1–40.0	42	213	5.1	.4	79	36
Hunter's Siding:						
0.1–5.0	172	20	.1	.1	6	5
5.1–10.0	189	42	.2	.1	16	7
10.1–15.0	194	85	.4	.1	21	7
15.1–20.0	108	60	.6	.2	19	8
20.1–25.0	43	19	.4	.1	33	12
Gold Center Creek:						
0.1–5.0	473	178	.4	.3	25	24
5.1–10.0	122	189	1.6	1.0	57	41
10.1–15.0	11	19	1.7	.6	73	45

[1] Infection originating in 1924 to 1928, inclusive.
[2] The plus sign signifies percentages greater than 0 but less than 0.5.

RELATIONSHIP BETWEEN INFECTION AND DAMAGE IN THE STAND AS A WHOLE

As pine infection pyramids in a stand, the percentage of trees infected increases more and more slowly relative to the increase in the number of cankers. For example, suppose that a stand exists in which all the trees are of the same size, are growing under the same environmental conditions, and are exposed to infection from distant concentrations of ribes. The resulting infection will not be absolutely uniform throughout the stand, but instead will be distributed almost at random; consequently, some of the trees will have more than one canker while others will have none. If n cankers originate and 40 percent of the trees become infected during the first year of exposure to the rust, and a second infection wave increases the number of cankers to $2n$, then the percentage of trees infected will not be increased to 80, but only to about 64. Cankers originating during the second wave will, it is true, infect approximately 40 percent of the trees, but on an average only three-fifths of this 40 percent will consist of trees becoming infected for the first time, since the infection is distributed at random through a stand in which two-fifths of the trees are already infected. In the same way, subsequent infection waves equal in magnitude to the first will successively increase the percentage of trees infected to about 78, 87, etc.

Natural stands are never composed entirely of trees of exactly the same size subjected to identical environmental conditions. In many cases, also, the infected ribes are located in or near the stand. Consequently, some of the trees are much more likely to become infected than are others, and infection is even less uniform than if it were distributed purely at random. During each successive infection wave cankers will become more numerous on those trees whose size and situation render them most liable to infection, while some of the other trees may remain rust-free for a considerable period even in localities where the disease is abundant.

Figure 3 [6] shows the probable percentages of infection corresponding to different average numbers of cankers per tree under conditions permitting perfectly random infection,[7] and under the conditions that governed the distribution of the 1927 infection on the Revelstoke

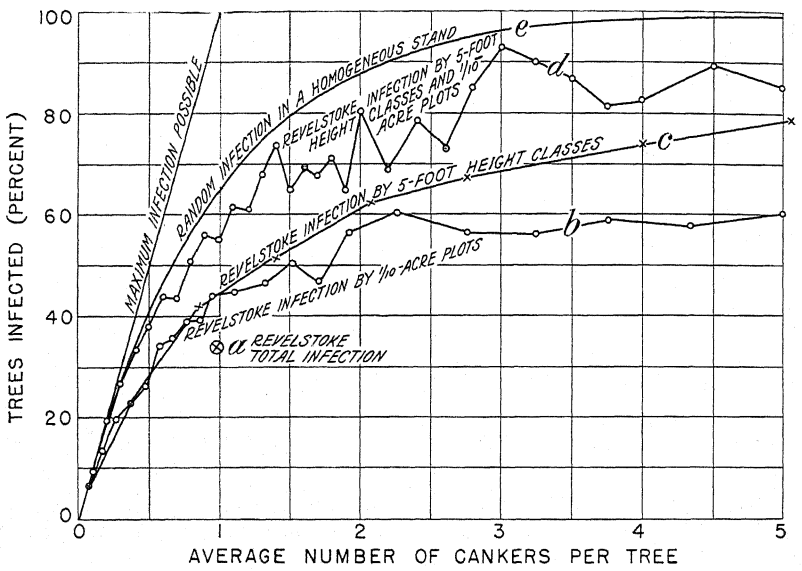


FIGURE 3.—Distribution of the 1927 infection on the Revelstoke area compared with random distribution. (Explanation in text.)

area. It will be seen that the percentage of infection on the Revelstoke area as a whole (point *a*) was far less than that which would have resulted from the same number of cankers distributed at random in a homogeneous stand of equal magnitude (curve *e*). In curve *b* the effect of differences in infection conditions (e. g., distance from ribes) has been reduced by dividing the area into ⅒-acre blocks, grouping the blocks according to the average number of cankers per tree

[6] Modified from: LACHMUND, H. G. DEGREE OF INFECTION BY CRONARTIUM RIBICOLA REQUIRED TO KILL OR SERIOUSLY INJURE TREES OF DIFFERENT SIZE CLASSES OF PINUS MONTICOLA. May 1933. [Unpublished manuscript.]

[7] This theoretical curve indicates infection probabilities in samples consisting of 10 trees each. This particular size of sample was chosen as a basis for the curve because it was of about the same general order of magnitude as most of the samples used in constructing curves *b* and *d* of fig. 3, and because it was convenient for calculation purposes. The form of the theoretical curve is affected to some extent by the size of sample for which it is drawn (e. g., where each sample consists of 10,000 trees, infection percentages are approximately 60, 84, 94, and 97 when the average numbers of cankers per tree are 1, 2, 3, and 4, respectively) but this variation is not sufficiently great to require consideration here.

(blocks containing less than four pines were not included in the computations), and plotting a separate point for each of the groups thus obtained. In curve *c* the effect of differences in tree size has been reduced by plotting a separate point for each 5-foot height class. In constructing curve *d*, separate computations were made for each 5-foot height class in each 1/10-acre block (blocks containing less than four pines of any given height class were not included in the computations for that particular class), and the subsequent grouping was determined entirely by canker frequency—i. e., the data were not first averaged by blocks. In this curve the effect of differences in both tree size and infection conditions has been appreciably reduced but has not been completely eliminated, since there may be relatively great differences in size between trees of a single 5-foot height class and since infection conditions may be far from uniform even within the limits of a 1/10-acre block (particularly when, as in the present instance, the ribes are located in or near the stand). Most, if not all, of the disparity between curve *d* and the theoretical curve (*e*) must therefore be ascribed to the diversity of tree sizes and situations characteristic of even the smallest units into which the basic data could practicably be organized.

For the sake of convenience the discussion in the three preceding paragraphs has been entirely confined to the relationship between total cankers present in a stand and the resultant percentage of trees infected. It is evident, however, that the same general relationship must exist between numbers of damaging cankers present and percentages of trees damaged. In the present instance, a smoothed curve drawn for the data presented in *d* of figure 3 differed algebraically by an average of only 0.14 percent from a curve constructed in the same way but based on numbers of damaging cankers and percentages of trees damaged in the various height classes and 1/10-acre blocks. Curve *c* of figure 3 may therefore be considered approximately indicative of the probable distribution of damage, under damaging-canker frequencies up to an average of five per tree, in any 5-foot height class on the Revelstoke area as a whole. From these premises it is possible to estimate the approximate extent of the damage likely to be caused on this area by different given numbers of cankers. Experience with the rust at numerous infection centers has shown that waves of pine infection become progressively more severe as the increasing abundance of aeciospores results in increased ribes infection and consequent production of telia. More than 10,000 cankers became established on the Revelstoke area within relatively few years after the first appearance of the disease in this locality, and during a period when production of telia was still far below the average capacity of *Ribes lacustre* (*6*); it therefore seems entirely possible that 100,000 or more cankers might originate here in a single year during the next one or two decades even if there were no increase in the size and number of the pines. One such wave, if distributed in the same way as the 1927 infection (table 4), would result in an average of about 0.5 damaging canker per tree in the 0.1- to 5.0-foot height class and more than 4.0 damaging cankers per tree in the 25.1- to 40.0-foot height classes. Curve *c* in figure 3 indicates that the resulting damage would involve more than 25 percent of the 0.1- to 5.0-foot trees and more than 70 percent of the 25.1- to 40.0-foot trees. It is obvious

that stands of young western white pine exposed to successive infection waves of even moderate severity will be ruined for all practical purposes before commercial maturity is attained.

SUMMARY

Western white pines on several areas in British Columbia and Idaho were carefully examined for infection. A calculation, based on previous studies of the behavior of the rust on pines, was made of the probable effect on the tree of each canker. The percentage of cankers capable of damaging was found to range from nearly 100 in the smallest trees to less than 30 in trees from 45 to 50 feet tall, but the larger trees (up to at least 40 feet in height) become much more heavily infected than do small trees in the same stand and are consequently more liable to damage. Within a given height class, the percentage of cankers capable of damaging and the length of time required for such damage to occur may vary, depending on crown width, rate at which branches are being killed by suppression, and other factors.

Although complete destruction of extensive young stands can occur only when enormous numbers of cankers are present, serious damage may result from relatively few cankers, and stands exposed to even moderately severe infection will be practically destroyed before becoming commercially mature.

LITERATURE CITED

(1) BUCHANAN, T. S.
1937. BLISTER RUST DAMAGE TO MERCHANTABLE WESTERN WHITE PINE. Jour. Forestry 36: 321–328, illus.

(2) ——— and KIMMEY, J. W.
1938. INITIAL TESTS OF DISTANCE OF SPREAD TO AND INTENSITY OF INFECTION ON PINUS MONTICOLA BY CRONARTIUM RIBICOLA FROM RIBES LACUSTRE AND R. VISCOSISSIMUM. Jour. Agr. Research 56: 9–30, illus.

(3) LACHMUND, H. G.
1933. METHOD OF DETERMINING AGE OF BLISTER RUST INFECTION ON WESTERN WHITE PINE. Jour. Agr. Research 46: 675–693, illus.

(4) ———
1934. GROWTH AND INJURIOUS EFFECTS OF CRONARTIUM RIBICOLA CANKERS ON PINUS MONTICOLA. Jour. Agr. Research 48: 475–503, illus.

(5) ———
1934. DAMAGE TO PINUS MONTICOLA BY CRONARTIUM RIBICOLA AT GARIBALDI, BRITISH COLUMBIA. Jour. Agr. Research 49: 239–249, illus.

(6) MIELKE, J. L.
1937. AN EXAMPLE OF THE ABILITY OF RIBES LACUSTRE TO INTENSIFY CRONARTIUM RIBICOLA ON PINUS MONTICOLA. Jour. Agr. Research 55: 873–882, illus.

(7) SNELL, WALTER H.
1928. BLISTER RUST IN THE ADIRONDACKS. Jour. Forestry 26: 472–486.

(8) ———
1929. SOME OBSERVATIONS OF THE WHITE PINE BLISTER RUST IN NEW YORK. Phytopathology 19: 269–283, illus.

(9) ———
1931. FOREST DAMAGE AND THE WHITE PINE BLISTER RUST. Jour. Forestry 29: 68–78.

(10) ———
1931. THE KELM MOUNTAIN BLISTER-RUST INFESTATION. Phytopathology 21: 919–921.

THE ESSENTIALITY OF GALLIUM TO GROWTH AND REPRODUCTION OF ASPERGILLUS NIGER[1]

By ROBERT A. STEINBERG

Associate physiologist, Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

In pursuance of the same general objectives as in previous work with *Aspergillus niger* Van Tiegh.,[2] the study of the growth of this organism with highly purified nutrient components has been continued. It has resulted in the identification of the chemical element gallium as essential for the nutrition and reproduction of this fungus.

The element gallium, so far as is known to the writer, has never been claimed to be essential to the growth of an organism. Though studies of its action on growth are to be found, the experimental conditions presumably were not such as to permit any evidence of its necessity to become known.

EXPERIMENTAL METHODS

The procedures employed in previous investigations were used in these studies with certain modifications. These changes consisted in the substitution of transparent quartz vessels for similar vessels of pyrex glass. The nutrient solutions were prepared with water distilled successively in an Acree metal still, a pyrex glass still, and a transparent quartz still. The sucrose, which contained 0.0014 percent ash, was extracted with 95-percent alcohol for 6 hours in a pyrex Soxhlet extractor before use. The chemicals used were practically spectroscopically pure, usually containing only very slight impurities of sodium, calcium, and iron.

Other conditions remained unchanged. The fungus *Aspergillus niger*, "W" strain, was grown on 50 cc of a 5-percent sugar solution to which all necessary salts had been added in approximately optimum concentrations. Inoculation was made with a spore suspension. Duration of growth was 4 days at 35° C., and the cultures were filtered through 1G3 Jena glass filters. The mycelial felts were weighed after drying overnight at 103° C.

EXPERIMENTAL RESULTS

The experimental results obtained under the conditions just described are shown in table 1. Experiment 1 gave a maximum yield of 847.1 mg under conditions apparently optimum, whereas with unextracted sucrose and reagent chemicals the yield was usually approximately 1,150 mg. Tests with salts of 77 of the chemical elements led to the selection of gallium as the element capable of eliminating the decrease in yield most efficiently. Only 0.02 mg of gallium

[1] Received for publication April 11, 1938; issued November 1938.

[2] STEINBERG, ROBERT A. ROLE OF MOLYBDENUM IN THE UTILIZATION OF AMMONIUM AND NITRATE NITROGEN BY ASPERGILLUS NIGER. Jour. Agr. Research 55: 891-902. 1937.

per liter was used in experiment 2. The maximum total yield reached a value of 1,123.6 mg, or almost exactly that obtained in many previous experiments with chemicals of slightly lower purity.

The next two experiments differed only as concerned the concentrations of the trace elements added to the nutrient solutions. In the third experiment, manganese was increased to 0.05 mg per liter, and in the fourth manganese and gallium were used at 0.05 and 0.01 mg per liter, respectively. The last two experiments of this table give the results first obtained with nutrient-solution purification by use of spectroscopically pure calcium and magnesium oxides.

TABLE 1.—*Trace-element requirements of Aspergillus niger when grown for 4 days at 35° C. in quartz vessels in a nutrient solution* [1] *made with triply distilled water, extracted sucrose, and spectroscopically pure chemicals*

Element omitted	Optimum concentrations of all known constituents				Gallium added to solution				Approximately optimum concentrations of all constituents			
	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation [2]	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation [2]	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation [2]
	Mg	*Per cent*	*pH*		*Mg*	*Per cent*	*pH*		*Mg*	*Per cent*	*pH*	
Fe	209.7	25.76	1.73	3, bl.	409.4	38.86	1.95	4, bl.	529.1	46.57	1.82	6, bl.
Zn	20.7	2.55	2.81	2, bl.	24.0	2.28	2.67	2, bl.	25.1	2.22	2.67	2, bl.
Cu	653.1	80.24	1.71	2, t.	892.1	84.67	1.55	2, bl.	991.7	87.63	1.54	2, bl.
Mn	339.6	41.73	1.79	0	398.3	37.80	1.70	0	363.7	32.14	1.74	0
Mo	747.4	91.82	1.64	4, bl.	1,014.1	96.25	1.52	4, bl.	1,134.1	100.21	1.52	3, bl.
Ga					935.5	88.79	1.56	5, bl.	968.1	85.54	1.55	8, bl.
None	814.0	100.00	1.77	7, bl.	1,053.6	100.00	1.56	4, bl.	1,131.7	100.00	1.57	4, bl.
Max.[3]	847.1				1,123.6				1,160.9			
C. U.[4]		33.88				44.94				46.44		
pH [5]			4.85				4.94				5.00	

Element omitted	Almost optimum concentrations of all constituents				Solution purified with CaO at 100° C.				Solution purified with MgO at 100° C.			
	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation [2]	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation [2]	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation [2]
	Mg	*Per cent*	*pH*		*Mg*	*Per cent*	*pH*		*Mg*	*Per cent*	*pH*	
Fe	44.36	42.05	1.89	6, bl.	80.2	12.79	2.76	1, bl.	592.3	71.14	1.87	4, bl.
Zn	24.3	2.30	2.75	2, bl.	221.8	35.38	2.31	10, bl.	38.0	4.57	2.81	4, bl.
Cu	998.0	94.62	1.55	2, br.	498.6	79.53	1.94	1, bl.	781.6	91.29	1.67	4, bl.
Mn	343.2	32.54	1.80	0	195.8	31.23	2.05	0	504.2	60.57	1.76	0
Mo	1,036.5	98.26	1.53	6, bl.	660.4	105.34	1.80	8, bl.	911.2	109.45	1.65	4, bl.
Ga	848.9	80.47	1.59	6, bl.	260.0	41.47	2.30	4, bl.	658.8	79.13	1.85	4, bl.
None	1,054.8	100.00	1.60	7, bl.	626.9	100.00	1.86	6, bl.	850.5	100.00	1.73	4, bl.
Max.[3]	1,161.7				1,008.8				1,145.1			
C. U.[4]		46.47				40.35				45.80		
pH [5]			4.90				6.86				6.43	

[1] Each liter of nutrient solution contained the following ingredients: Sucrose, 50.0 g; $(NH_4)_2SO_4$, 3.40 g; KH_2PO_4, 0.55 g; Mg (as chloride), 0.025 g; iron, 0.20 mg; zinc, 0.20 mg; copper, 0.05 mg; manganese, 0.025 mg; and molybdenum, 0.02 mg. Somewhat higher concentrations of salts were used in the purification experiments.

[2] Sporulation was estimated on a scale ranging from 0 (= sterility) to 10 (= maximum sporulation); color of spores is indicated by abbreviation of the words tan, brown, and black.

[3] Maximum individual yield.

[4] Coefficient of utilization, or yield per 100 g of sucrose.

[5] Initial acidity of the nutrient solution.

TABLE 1.—*Trace-element requirements of Aspergillus niger when grown for 4 days at 35° C. in quartz vessels in a nutrient solution made with triply distilled water, extracted sucrose, and spectroscopically pure chemicals*—Continued

Element omitted	Solution unpurified				Solution purified with CaO in steamer at 100° C. and filtered				Solution purified with CaO in autoclave at 120.5° C. and filtered			
	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation [2]	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation [2]	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation [2]
	Mg	*Per cent*	*pH*		*Mg*	*Per cent*	*pH*		*Mg*	*Per cent*	*pH*	
Fe	526.5	45.26	1.81	6, bl.	72.2	8.13	2.55	1, bl.	123.8	12.31	2.36	2, bl.
Zn	22.6	1.94	2.76	2, bl.	74.9	8.43	2.59	4, bl.	153.1	15.23	2.31	8, bl.
Cu	1,056.1	90.78	1.56	2, br.	461.1	51.94	1.95	2, bl.	596.3	59.30	1.75	2, br.
Mn	369.6	31.77	1.77	0	241.5	26.07	1.94	0	221.0	21.98	2.54	0
Mo	1,061.1	91.21	1.53	4, bl.	861.6	97.03	1.63	6, bl.	1,043.2	103.75	1.51	8, bl.
Ga	926.5	79.64	1.58	4, bl.	334.6	37.68	2.17	4, bl.	431.2	42.89	2.06	5, bl.
None	1,163.3	100.00	1.54	4, bl.	887.9	100.00	1.63	6, bl.	1,005.5	100.00	1.58	8, bl.
Max.[3]	1,167.8				1,101.7				1,236.0			
C. U.[4]		46.71				44.07				49.44		
ph [5]			4.67				6.64				6.49	

[2] Sporulation was estimated on a scale ranging from 0 (= sterility) to 10 (= maximum sporulation); color of spores is indicated by abbreviation of the words tan, brown, and black.
[3] Maximum individual yield.
[4] Coefficient of utilization, or yield per 100 g of sucrose.
[5] Initial acidity of the nutrient solution.

The results of deficiency tests with zinc and manganese were quite good, those with copper average, and those with iron and molybdenum poor. Purification was of little effect except with gallium. Acidities at harvest differed somewhat from those obtained in previous investigations with reagent chemicals. The acidities of the minus-manganese cultures no longer tend to be higher than those of the control and the other minus cultures, though this may be associated with the exceptionally low yields. The effects of low gallium on reproduction were not marked, and seemed analogous to those of iron and zinc with respect to the extent of deficiency necessary to affect sporulation. Other untabulated experiments have demonstrated that moderate increases in iron, zinc, copper, manganese, or molybdenum were unable to overcome a deficiency in gallium.

A comparison of the effects of trace-element deficiencies on the growth of *Aspergillus* in an unpurified solution when heated at 100° C. in the steamer and when treated at 120.5° in the autoclave is given in table 1. The deficiency data are quite similar to those obtained in the previous experiments. Heating at the higher temperature did not improve the degree to which the trace elements were removed.

Several features deserve mention. The results with iron are poor despite the use of spectroscopically pure compounds, but show marked improvement as a consequence of nutrient-solution purification. Zinc results were the best yet obtained without purification, whereas purified solutions with spectroscopically pure compounds gave poorer results than purification with reagent compounds. Manganese-deficiency results likewise were superior to any yet obtained. The response to molybdenum deficiency even under these conditions is quite slight and in entire conformity with the special needs of *Asper-*

TABLE 2.—*Effect of various nitrogen compounds on trace-element requirements of Aspergillus niger when grown for 4 days at 35° C. in pyrex glass flasks in a nutrient solution* [1] *made with water distilled in pyrex glass, extracted sucrose, and spectroscopically pure trace elements*

Element omitted	$(NH_4)_2SO_4$				NH_4Cl (solution purified with CaO)				Urea				$NaNO_3$ (solution purified with $CaCO_3$)				NH_4Cl (solution purified with $CaCO_3$)				Urea			
	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation [1]	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation
	Mg	*Per cent*	*pH*		*Mg*	*Per cent*	*pH*		*Mg*	*Per cent*	*pH*		*Mg*	*Per cent*	*pH*		*Mg*	*Per cent*	*pH*		*Mg*	*Per cent*	*pH*	
Fe	308.6	31.98	2.02	6, bl.	11.4	1.51	2.74	1, bl.	154.6	20.30	2.62	1, bl.	12.7	1.46	3.16	1, bl.	12.1	1.03	2.87	0	198.2	33.21	2.69	2, bl.
Zn	30.1	3.12	2.73	2, bl.	41.5	5.50	2.70	1, bl.	136.2	17.88	2.68	3, bl.	17.1	1.96	3.31	1, bl.	20.2	1.73	2.89	0	29.2	4.90	2.77	2, bl.
Cu	804.2	83.24	1.60	6, br.	606.4	80.36	1.58	2, bl.	497.9	65.39	2.51	3, br.	614.3	70.46	3.15	4, t.	1,051.2	90.07	1.40	1, bl.	344.8	57.77	2.74	2, bl.
Mn	349.6	36.19	1.71	0	136.0	25.58	1.93	0	778.4	102.24	2.07	6, bl.	700.5	80.35	2.01	1, bl.	806.8	69.07	1.40	2, bl.	371.8	62.27	2.77	2, bl.
Mo	856.4	88.65	1.56	8, bl.	562.1	74.50	1.56	2, bl.	746.2	98.01	2.30	10, bl.	942.0	108.05	2.66	8, bl.	1,151.5	98.58	1.38	2, bl.	374.9	62.79	2.70	6, bl.
Ba	704.9	72.96	1.67	8, bl.	664.9	88.12	1.54	2, bl.	622.5	81.76	2.56	8, bl.	844.4	96.86	2.86	6, br.	1,052.8	90.13	1.38	2, bl.	232.5	38.96	2.74	2, bl.
None	966.1	100.00	1.53	7, bl.	754.5	100.00	1.49	4, bl.	761.4	100.00	2.31	10, bl.	871.8	100.00	2.89	10, bl.	1,168.1	100.00	1.40	2, bl.	596.7	100.00	2.43	8, bl.
Max.[2]	997.6				911.4				920.0				1,017.9				1,221.7				697.3			
C. U.[3]		39.90				36.46				36.80				40.72				48.87				27.89		
pH [4]			5.23				7.48				7.43				7.08				6.94				7.47	

[1] See footnote 2, table 1.
[2] See footnote 3, table 1.
[3] See footnote 4, table 1.
[4] See footnote 5, table 1.

gillus for this element with nitrate nitrogen [3] as compared with ammonium nitrogen.

A few miscellaneous experiments are shown in table 2. They were performed in the course of work now in progress to determine the extent to which quartz ware and spectroscopically pure chemicals might be dispensed with in studies on gallium. Pyrex ware and reagent chemicals, for example, were used in the experiment with ammonium sulphate together with extracted sucrose, spectroscopically pure monopotassium phosphate, and spectroscopically pure trace elements. Other experiments were performed under similar conditions except that reagent dipotassium phosphate was used. In the first experiment with urea nitrogen only reagent chemicals and unextracted sucrose were employed. Only spectroscopically pure calcium oxide was used for purification of the nutrient solution. Even these preliminary data, nevertheless, show the marked influence of gallium on the growth of *Aspergillus* under a wide range of experimental conditions. Under optimum conditions for growth the customary improvement in quantitative results should also be forthcoming.

DISCUSSION

The evidence obtained in favor of the essentiality of gallium for *Aspergillus* is quite definite, similar results having been obtained under a variety of conditions. Spectroscopically pure gallium as chloride in concentrations of 10 to 30 parts per billion aids both growth and sporulation. Salts of none of the other 76 chemical elements tested were effective to this degree at comparable concentrations. Its action, in other words, appears to be specific. The use of quartz ware and of spectroscopically pure sources of nitrogen, potassium, phosphorus, magnesium, and sulphur does not appear an essential prerequisite for positive results. Nevertheless, the use of pyrex glass vessels and reagent chemicals, even if spectroscopically pure trace elements and calcium oxide and extracted sucrose were employed, gave appreciably poorer results on gallium. The extent of the loss in yield accompanying the omission of gallium from the nutrient solution surpassed that obtained through the omission of copper, however.

The evidence obtained, therefore, in favor of the view that gallium is an essential element for *Aspergillus* is positive as respects specificity and favorable influence on growth and reproduction under a wide range of conditions. The purity of the gallium salts employed in these tests, together with the very low concentrations employed would also tend to substantiate this interpretation. That is, every known test for biological essentiality has given positive results.

The extension of these results to another organism would also be particularly desirable. Certain outstanding facts may serve as a guide in proving the necessity of gallium to other organisms. Gallium[4] is quite similar in chemical properties to aluminum and has frequently been reported as aluminum in quantitative determinations. It is widely distributed in nature in small quantities. Its absence as an impurity in aluminum salts is by no means certain, nor is its nonresponsibility for the frequently reported biologically beneficial action

[3] STEINBERG, R. A. See footnote 2.

of aluminum.[5] Some of the effects of the latter can be attributed to acidity alone, however. The reported toxicity of thallium, a chemical homologue of gallium, to tobacco by McMurtrey,[6] in association with the close duplication of the symptoms of frenching, would appear to indicate the possibility that thallium prevented the utilization of gallium by the plant. Moreover, the conditions under which this disease of the tobacco plant occurs are quite consistent with the behavior of gallium salts. A similar relation between the effects of sulphur and selenium on green plants has been reported by Hurd-Karrer.[7] The beneficial action of thallium on yeast reported by Richards[8] may also have been due to the partial substitution of thallium for the gallium needs of yeast, a well-known type of deficiency phenomenon among the fungi.

SUMMARY

Aspergillus niger was grown in quartz Erlenmeyer flasks at 35° C. for 4 days in nutrient solutions composed of sucrose extracted with alcohol; water distilled successively in an Acree metal still, a pyrex glass still, and a quartz still; and spectroscopically pure salts. The element gallium was identified as essential to the growth and development of this organism at concentrations of 0.01 to 0.02 mg per liter. Yields as low as 38 percent of maximum were obtained on its omission from the cultures. Gallium was necessary, apparently, in addition to iron, zinc, copper, manganese, and molybdenum.

[4] HILLEBRAND, W. F., and LUNDELL, G. E. F. APPLIED INORGANIC ANALYSIS; WITH SPECIAL REFERENCE TO THE ANALYSIS OF METALS, MINERALS AND ROCKS. 929 pp., illus. New York and London. 1929.

[5] SPENCER, ERNEST L. FRENCHING OF TOBACCO AND THALLIUM TOXICITY. Amer. Jour. Bot. 24: 16-24. illus. 1937.

[6] MCMURTREY, J. E., JR. EFFECT OF THALLIUM ON GROWTH OF TOBACCO PLANTS. Science 76: 86. 1932.

[7] HURD-KARRER, ANNIE M. INHIBITION OF SELENIUM INJURY TO WHEAT PLANTS BY SULFUR. Science 78: 560. 1933.

[8] RICHARDS, OSCAR W. THE STIMULATION OF YEAST GROWTH BY THALLIUM, A "BIOS" IMPURITY OF ASPARAGINE. Jour. Biol. Chem. 96: 405-418, illus. 1932.

TIME INTERVAL BETWEEN CLUTCHES IN RHODE ISLAND RED PULLETS[1]

By F. A. HAYS

Research professor, Department of Poultry Husbandry, Massachusetts Agricultural Experiment Station

INTRODUCTION

This paper supplements one published in 1936[2] on the same group of birds. In the first report consideration was given to the mean time interval between eggs within the clutch for the winter season and for the first laying year. This paper presents the results of a study of the mean time interval between clutches in relation to time between eggs of the clutch, clutch size, winter production, hatching-season egg weight, hatchability, persistency, and annual production.

In the previous paper the term "clutch" was used to include eggs laid on successive days, and the range in clutch size was found to vary widely in the population studied. In the work here reported a study was made of the interval between clutches throughout the first laying year. Clutches that make up any particular cycle of eggs are separated by less than 48 hours, but the interval between the last egg of a cycle and the first egg of the next cycle may be greater than 48 hours. No specific evidence is available concerning the causes of intervals of 2 to 7 days between litters in winter or of intervals of less than 30 days in spring and summer. Necessarily a rather liberal use of interval between clutches must be proposed.

CHANGES IN TIME INTERVAL BETWEEN CLUTCHES DURING THE PULLET LAYING YEAR

In calculating the time interval between clutches the cessation of laying in the birds that exhibit winter pause creates a difficulty. Since evidence had been obtained[3] that in the stock used pauses of shorter duration than 8 days are not inherited but pauses of 8 or more days are likely to depend on inheritance, it was considered advisable to omit all winter pauses of 8 days or more from November 1 to March 1. After March 1, pauses of 30 days or more probably mark the termination of the biological year and these have been omitted. All broody pauses are also omitted. The data as presented on mean monthly time interval between clutches are an approximation of normal egg laying in birds free from winter pause and in birds not undergoing annual molt within the first laying year.

[1] Received for publication February 26, 1938; issued November 1938. Contribution No. 307 of the Massachusetts Agricultural Experiment Station.

[2] HAYS, F. A. TIME INTERVAL BETWEEN EGGS OF RHODE ISLAND RED PULLETS. Jour. Agr. Research 52: 633–638, illus. 1936.

[3] HAYS, F. A. WINTER PAUSE IN RHODE ISLAND REDS. Mass. Agr. Expt. Sta. Bull. 329, 11 pp., illus. 1936.

Figure 1 indicates that pullets that are getting well started on their laying year in October are likely to lay with an interval of about 66 hours between clutches. In November and again in December there was a rapid decline in the interval between clutches. During December the shortest interval between clutches occurred. From the short interval of about 48 hours during December there was a gradual lengthening of the interval up to May. During May the interval took a very abrupt increase to about 64 hours. During June the interval showed another abrupt increase over May to about 96 hours. During July there was a slight decline to an interval of about 94 hours. In August the interval between clutches declined to 89 hours, and in

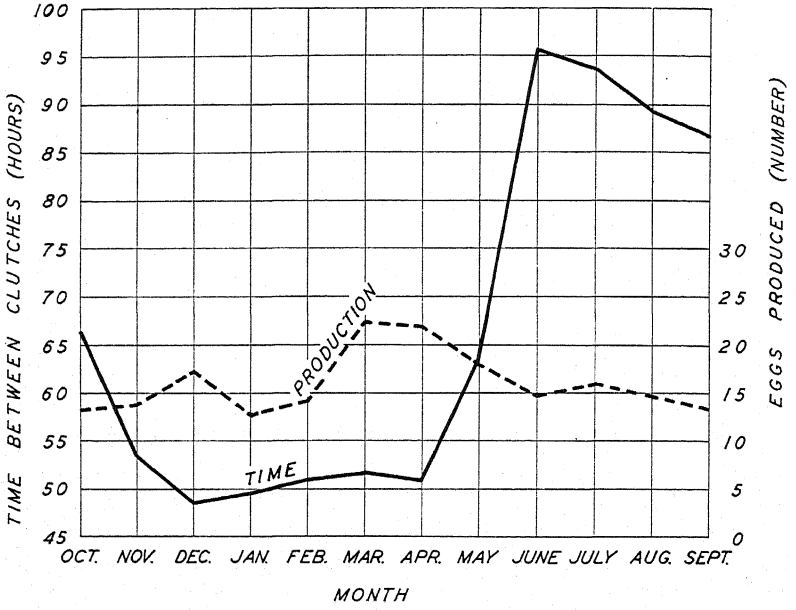


FIGURE 1.—Relation between monthly egg production and monthly interval between clutches, based on the records of 119 birds, pauses of over 8 days from November 1 to March 1 and all broody periods omitted.

September, which represents the last month of the first laying year, the interval was 87 hours.

These data show in general that when winter pause was excluded the birds were likely to lay with an interval of about 2 days between clutches during December, January, February, and March. The spring and summer seasons appeared to be characterized by intervals of 3 or 4 days between clutches. Heavy egg production and short intervals between clutches during the winter appeared to be characteristic features of production-bred flocks. In the previous report the shortest interval between eggs within the clutch occurred in April. In the data now being considered the shortest interval between clutches occurred in December.

RELATION BETWEEN MONTHLY INTERVAL BETWEEN CLUTCHES AND MONTHLY INTERVAL BETWEEN EGGS

It is desirable to know the relationship of the time interval between eggs within the clutches. These data are recorded graphically in figure 2, which presents the mean monthly interval between clutches and the mean monthly interval between eggs for the first laying year. These graphs show that short intervals between clutches were generally associated with long intervals between eggs within the clutches. For example, in February when the interval between clutches was

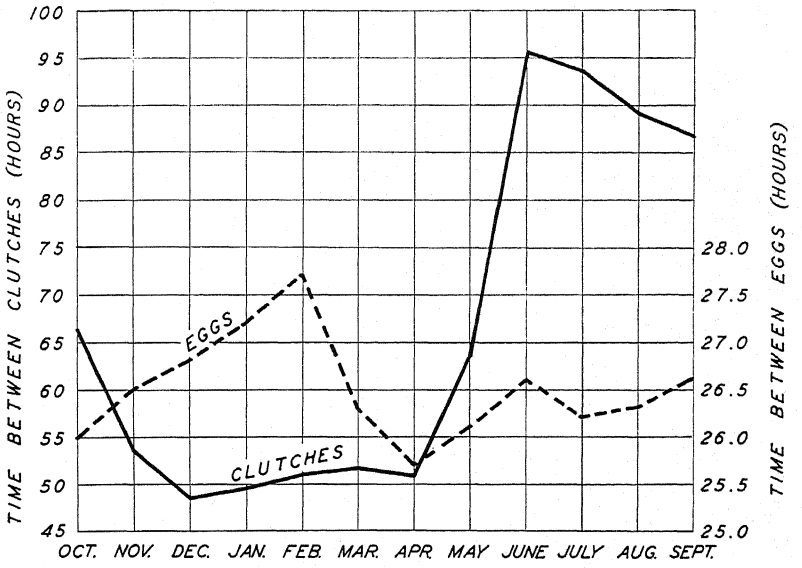


FIGURE 2.—Relation between monthly interval between clutches and monthly interval between eggs based on the records of 119 birds.

very short, the interval between eggs within the clutch reached the high level for the year. In summer when the interval between clutches increased to its greatest length, the interval between eggs remained at a relatively low level. In March, April, and May both time intervals were very short. This particular spring period offers optimum conditions for egg laying by both improved and unimproved flocks. During the winter and during the summer months when conditions are further from optimum for very intense production, birds seemed to compensate a short interval between clutches by a long interval between the eggs of a clutch or a long interval between clutches by a short interval between the eggs of a clutch. These data suggest that a true measure of intensity would involve both the interval between clutches and the interval between eggs within the clutches.

INTERVAL BETWEEN CLUTCHES IN WINTER AS RELATED TO SIZE OF WINTER CLUTCH

Data are available on 409 birds including those with winter pause and those without winter pause. After considerable study it was decided to calculate the time interval between remaining clutches after eliminating all pauses of 8 days or more between November 1 and March 1. By this method the population of 409 individuals did not approach a normal frequency distribution. In figure 3 the population is divided into nonpause and pause groups on the 8-day basis. There were 142 nonpause birds and 267 pause birds. Figure 3 indi-

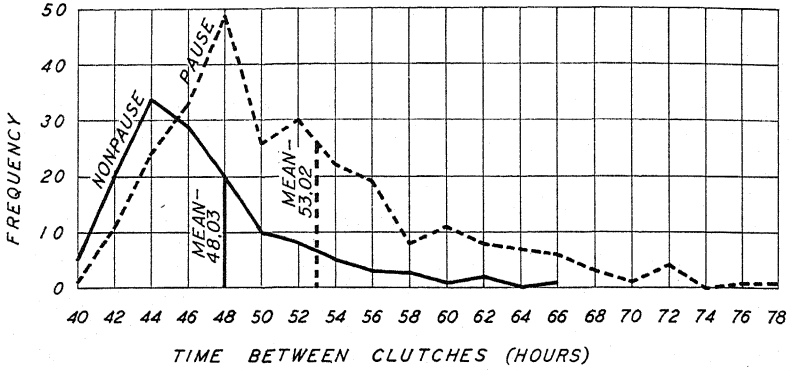


FIGURE 3.—Frequency distribution of time between clutches during winter (to March 1, 1927) for 142 nonpause birds and 267 pause birds. All pauses of 8 or more days between November 1 and March 1 are omitted in calculating time between clutches.

cates that nonpause birds required less time between clutches in the winter season than pause birds. There appeared to be two distributions with considerable overlapping, but neither distribution was sufficiently close to normal to be treated as such. Birds with winter pause were more likely to exhibit a greater time interval between clutches when they were actively laying than were nonpause birds. The variability in time interval between clutches was extremely high as compared with the variability in time interval between eggs of a clutch, as noted in the previous report.

Table 1 indicates a downward trend in clutch size with an increasing time interval between clutches in the winter season. This decline was regular and consistent until the time interval between clutches approached 65.5 hours. This particular group of 11 birds showed an unusually large mean clutch size because of three individuals that had a winter clutch size of from 5.5 to 6.5 eggs. These data therefore appear to justify the deduction that mean time intervals greater than 54 or 55 hours between clutches were likely to be associated with small clutch size or low intensity.

INTERVAL BETWEEN CLUTCHES IN WINTER AS RELATED TO WINTER EGG PRODUCTION

Mean egg production from first egg to March 1 showed generally a very consistent decline as the interval between clutches increased.

The group of 11 birds in the 65.5-hour class was an exception because of the very high winter egg record of three individuals. These data suggest that short intervals between clutches are essential to heavy winter laying.

TABLE 1.—*Time between clutches as related to winter clutch size and winter production in 394 birds*

Time between clutches in winter (hours)	Birds	Egg clutch size in winter	Winter egg production	Time between clutches in winter (hours)	Birds	Egg clutch size in winter	Winter egg production
	Number	*Number*	*Number*		*Number*	*Number*	*Number*
41.5	19	3.56	69.71	65.5	11	2.90	59.14
44.5	75	2.96	66.70	68.5	5	1.65	37.50
47.5	99	2.74	64.79	71.5	2	6.95	70.50
50.5	63	2.83	64.87	74.5	1	1.45	15.50
53.5	53	2.68	59.08	77.5	1	1.45	15.50
56.5	32	2.54	55.19	80.5	1	2.45	85.50
59.5	17	2.39	55.50	83.5	0		
62.5	13	2.30	47.04	86.5	2	2.45	25.50

INTERVAL BETWEEN CLUTCHES IN WINTER AS RELATED TO HATCHING-SEASON EGG WEIGHT AND HATCHABILITY

There were 47 individuals that were used for breeding in the spring of 1927. Their eggs were weighed during the last week of February and the first week of March to secure an approximation of the egg weight of each bird during the hatching season extending from February 22 to April 22.

TABLE 2.—*Time interval between clutches as related to egg weight during hatching season and to hatchability of eggs from 47 birds used for breeding*

Time between clutches in winter (hours)	Birds	Egg weight during hatching season	Hatchability of eggs	Time between clutches in winter (hours)	Birds	Egg weight during hatching season	Hatchability of eggs
	Number	*Grams*	*Percent*		*Number*	*Grams*	*Percent*
41.5	5	55.5	59.5	56.5	2	55.5	35.5
44.5	15	54.8	61.9	59.6	0		
47.5	8	53.5	40.9	62.5	0		
50.5	8	52.0	47.3	65.5	2	53.5	27.8
53.5	7	53.4	56.9				

The data in table 2 show little relationship between the time interval between clutches up to March 1 and egg weight taken about March 1. There was, however, a possible suggestion of a slight decline in egg weight associated with an increased time interval between clutches.

The relation of time between clutches up to March 1 and the percentage of eggs that hatched is shown in table 2. The highest hatchability occurred in the group of 15 birds with a mean time interval of 44.5 hours between clutches. The group with the shorter interval of 41.5 hours also ranked high in hatchability. As the interval lengthened there was a tendency for hatchability to decline. Although the data are limited, there is some evidence to indicate that birds producing clutches of eggs at frequent intervals are likely to produce eggs of high hatchability.

INTERVAL BETWEEN CLUTCHES IN WINTER AS RELATED TO PERSISTENCY AND ANNUAL PRODUCTION

Persistency records covering the 365-day laying year are available on 245 birds. The data in table 3 indicate no relationship between the time interval between clutches and the duration of the laying year previous to the annual molt. The two groups of birds with a mean interval between clutches ranging from 64 to 70 hours exhibited unusually high persistency but were represented by only nine individuals and can be considered of no particular significance.

TABLE 3.—*Time between clutches as related to annual persistency and annual egg production of 245 birds*

Time between clutches during winter (hours)	Birds	Annual persistency	Annual egg production	Time between clutches during winter (hours)	Birds	Annual persistency	Annual egg production
	Number	*Days*	*Number*		*Number*	*Days*	*Number*
41.5	13	332.5	230.7	56.5	13	331.3	193.0
44.5	59	322.1	215.0	59.5	9	342.3	176.7
47.5	62	322.5	206.6	62.5	5	308.0	162.5
50.5	43	331.1	200.6	65.5	5	356.0	216.5
53.5	32	332.8	197.0	68.5	4	355.3	209.5

Annual egg production showed a definite downward trend as the time interval between clutches increased. The small group of nine birds at the bottom of the table made reasonably good annual egg records because of their very high persistency. The data in table 3 further confirm the common observation that for maximum annual records a maximum of time spent in productive laying during the winter season is essential.

SUMMARY

The mean time interval between clutches was calculated on 142 nonpause birds and on 267 pause birds up to March 1. Monthly time intervals between clutches were determined on 119 of these birds that were housed together. The relation of time interval between clutches to a number of fecundity and reproductive characters was studied. The significant relationships observed were as follows:

(1) Monthly interval between clutches attained its lowest level in December and remained at a low level from December through April. In May, June, and July the interval increased very rapidly and reached the highest level of the year in June.

(2) Although the mean time interval between clutches was short in winter after pauses had been eliminated, the level of egg production in January and February was about the same as from June to the end of the year because of winter pause.

(3) Birds with winter pause showed a greater mean time interval between clutches than nonpause birds when both were actually laying.

(4) Short intervals between clutches were associated with long intervals between eggs within the clutches, and vice versa.

(5) Small clutch size in winter was significantly associated with long time intervals between clutches.

(6) Short time intervals between clutches were associated with high winter production.

(7) There was no significant relationship between time interval between clutches and hatching-season egg weight.

(8) There was some evidence that short intervals between clutches in winter were associated with high hatchability.

(9) The time interval between clutches in winter showed no association with persistency at the close of the laying year.

(10) Short time intervals between clutches occurred in the high producers.

(11) In a breeding program short time intervals between clutches in winter should be placed along with short time intervals between eggs of a clutch as a desirable character.

RELATION BETWEEN FIBER LENGTH AND MATURITY IN COTTON [1]

By R. S. HAWKINS
Agronomist, Arizona Agricultural Experiment Station

INTRODUCTION

Plant breeders throughout the Cotton Belt are striving to increase both the length and the uniformity in length of cotton fibers. They are also interested in fiber improvement from the standpoint of maturity or wall thickness. This latter quality is less easily discernible to the naked eye than are length determinations, and if it could be safely assumed that a significant correlation exists between length and maturity, cotton-fiber improvement work would be materially simplified. The data included in this paper relate to such a correlation.

MATERIAL AND METHODS

Thirty-one samples of seed cotton were collected from as many locations in the upper Gila Valley in Arizona on October 5, 1937. This area is strictly a one-variety community growing Acala cotton. Varying availability of irrigation supplies caused wide differences in plant types, ranging from dwarfed, water-stressed plants to over-vegetative plants. The two extremes are illustrated in figure 1. One lock from each of 50 representative bolls constituted a field sample. The middle seed of each of 10 locks was placed in a Pressley sorter [2] and the percentages of fibers of various lengths were determined in the usual way. Duplicate samples were sorted in each case, making a total of 20 seeds from each field sample. In the interest of economy of time, the length classes were grouped as indicated in table 1. Such grouping probably resulted in automatically smoothing the curves in figure 2 and in making the data less variable than would have been the case if more length classes had been used.

Fiber maturity in each length class was obtained by the Shirley Institute method [3] in which the mature, intermediate, and immature fibers are counted under the microscope after treatment with an 18-percent aqueous solution of sodium hydroxide. Four samples of 100 fibers each were counted from each length class and also from the combings from each sample.

To convert the data concerning fiber maturity into a single factor, the formula $MI=\frac{10m+5t+1i}{N}$ was used. MI represents the maturity index, m the number of mature fibers in the sample, t the number of fibers intermediate in maturity, i the immature fibers, and N the total

[1] Received for publication February 16, 1938; issued November 1938.
[2] PRESSLEY, E. H. A NEW TYPE OF COTTON SORTER. Jour. Amer. Soc. Agron. 25: 89-98, illus. 1933.
[3] CLEGG, G. G. IMMATURITY OF COTTON. Empire Cotton Growing Corp. Conf. on Cotton Growing Problems, Rept. and Summary Proc. August 1930; 13-17. 1930.

number of fibers in the sample. The formula is based upon data reported by Hawkins and Serviss [4] in which it was determined that the walls of mature Acala and Pima fibers are approximately 10 times as thick as those of immature fibers. Since all gradations in fiber-wall thickness exist in the intermediate fibers, it is assumed that these





FIGURE 1.—*A*, Typical field of water-stressed cotton plants; *B*, large, overirrigated vegetative plants.

fibers average midway between the mature and immature fibers in wall thickness. The thickening of fiber walls is a biological process effected by hereditary and environmental influences. The maturity index as used in this paper is a measure of these influences on the average thickness of the fiber walls in a given sample.

[4] HAWKINS, R. S., and SERVISS, GEORGE H. DEVELOPMENT OF FIBERS IN THE PIMA AND ACALA VARIETIES. Jour. Agr. Research 40: 1017–1029, illus. 1930.

EXPERIMENTAL RESULTS

The maturity indices for the fiber by length classes of the 31 samples of cotton, together with the percentages of fibers included in each length class, are given in table 1. A small proportion of the fibers becomes detached from the seed during the combing process prior to placement in the sorter. The length of fiber distribution made from combings indicates that usually the fiber lengths that make up the combings are approximately in the same proportion as in the sample proper. However, the combings contain more immature fibers than any of the length classes as shown by the data in table 1. This is not surprising, since immature fibers are weaker and more easily detached or broken than mature ones.

TABLE 1.—*Maturity indices and percentages of cotton fibers in relation to length classes*

Field sample No.	Combings [1]		Four-eighths and five-eighths inch		Six-eighths and seven-eighths inch		Eight-eighths and nine-eighths inches		Ten-eighths inches		Weighted average maturity index for sample
	Maturity index	Percent	Maturity index	Percent	Maturity index	Percent	Maturity index	Percent	Maturity index	Percent	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
1	0.277	31.4	0.251	21.3	0.375	19.0	0.383	25.6	0.403	2.8	0.321
2	.389	10.7	.545	9.3	.483	25.7	.584	48.4	.427	5.8	.524
3	.397	5.8	.462	10.2	.570	30.6	.611	48.8	.409	4.7	.562
4	.521	10.0	.510	10.3	.568	22.4	.581	49.9	.453	7.4	.553
5	.476	10.6	.507	10.0	.552	21.8	.654	52.1	.463	5.5	.588
6	.523	10.0	.583	11.6	.609	21.8	.631	51.5	.485	5.2	.603
7	.444	7.5	.561	9.4	.579	20.0	.536	55.7	.654	7.4	.549
8	.464	15.5	.468	13.3	.540	24.4	.529	38.3	.578	8.4	.517
9	.495	6.4	.594	11.2	.547	19.6	.617	55.3	.479	7.5	.583
10	.426	7.6	.578	14.2	.597	34.3	.602	41.1	.462	2.8	.580
11	.398	5.5	.570	14.9	.568	33.3	.602	42.0	.479	4.2	.569
12	.538	11.9	.515	14.8	.691	33.1	.629	37.7	.375	2.5	.615
13	.464	15.8	.515	16.4	.611	35.6	.646	30.9	.365	1.2	.579
14	.413	10.2	.543	10.5	.535	16.6	.393	55.8	.364	7.0	.433
15	.523	13.6	.562	15.2	.637	31.6	.684	37.8	.549	1.9	.627
16	.424	7.5	.549	12.3	.578	21.8	.519	50.5	.502	8.0	.528
17	.426	10.8	.469	8.6	.536	17.3	.568	54.6	.355	8.6	.520
18	.448	13.0	.632	17.7	.539	30.8	.523	33.4	.463	5.0	.534
19	.522	9.4	.601	12.7	.636	29.1	.715	44.1	.702	4.7	.659
20	.526	8.0	.559	9.6	.634	20.5	.634	54.1	.481	7.8	.602
21	.500	10.8	.591	10.1	.600	20.9	.622	53.0	.543	5.2	.597
22	.543	10.9	.583	9.4	.559	21.0	.646	52.9	.496	5.7	.601
23	.625	7.0	.562	10.7	.646	24.8	.580	52.9	.430	4.6	.591
24	.393	12.3	.538	12.5	.559	21.7	.518	46.6	.450	6.9	.509
25	.450	13.6	.502	13.7	.577	24.7	.567	42.8	.499	5.2	.541
26	.474	12.0	.589	13.5	.666	19.9	.611	48.6	.526	5.9	.597
27	.664	11.9	.522	9.2	.661	28.6	.644	42.3	.607	8.0	.623
28	.547	11.3	.516	11.6	.599	14.9	.635	49.9	.629	12.2	.605
29	.522	8.2	.607	11.0	.578	16.6	.725	55.8	.542	8.4	.656
30	.349	12.5	.450	15.5	.515	26.2	.436	38.9	.433	6.8	.474
31	.481	7.6	.639	13.3	.667	28.3	.617	43.7	.623	7.0	.624
Mean	.472	10.95	.538	12.39	.581	24.42	.588	46.29	.491	5.95	.563

Mean difference of the differences between (4) and (6), 0.060; between (6) and (8), 0.007; between (8) and (10), −0.094. and error of mean differences ±0.008 [2], ±0.011, and ±0.017 [2].

[1] Percentages are based on fibers four-eighths of an inch or longer, assuming that most of the shorter fibers go into waste.

[2] Highly significant differences, as calculated by Student's *t* method, the chances being greater than 99 to 1 that the differences are not due to chance. (See text for more detailed explanation.)

The longest fibers, those of the 1⅛-inch class, were more immature than fibers of any other class, having a low average maturity index of 0.491. However, this length class contained only 5.95 percent of the fibers, a materially lower percentage than any other length class.

The best fibers from the standpoint of maturity were in the ⅝- and ¾-inch class with a maturity index of 0.588, although there was no significant difference between this class and the ½ and ⅞-inch class, which had a maturity index of 0.581. The former class contained almost half of the total fibers, 46 percent, and these two classes comprised more than 70 percent of the total fibers. The shortest fibers considered, the ⅜- and ½-inch class, had a maturity index of 0.538, which was materially better than that of the longest fibers but inferior to that of the intermediate classes.

Statistical analysis by means of Student's *t* method, as described by Fisher,[5] shows that the differences in maturity between the short, intermediate, and long length classes are highly significant, the chances being greater than 99 to 1 that these differences are not due to chance.

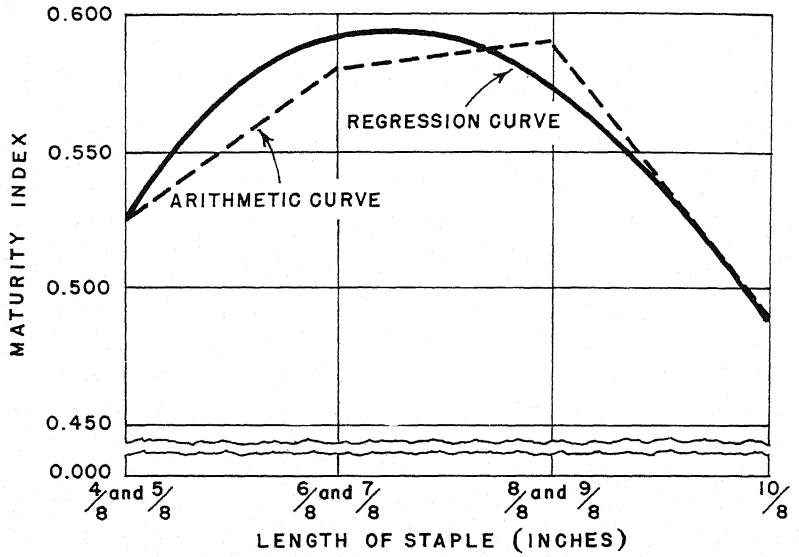


FIGURE 2.—Relation between maturity and length of staple as calculated from table 1. (Method described by LOVE, HARRY H. APPLICATIONS OF STATISTICAL METHODS TO AGRICULTURAL RESEARCH. 501 pp., illus. Shanghai. 1936.)

According to Student's *t* method, when the quotient of the mean differences divided by the error of the mean differences falls between 2.042 and 2.750 (with 31 samples involved as in this particular problem), the data show significant differences. No significance is shown when the quotient is lower than 2.042, and when higher than 2.750 the differences are highly significant. Thus the mean of the differences between the maturity indices in columns 4 and 6 is 0.060 and when divided by the error of the mean difference, 0.008, gives a quotient which is very much greater than 2.750 and therefore is highly significant. Similarly, the differences between columns 6 and 8 are not significant, while those between columns 8 and 10 are highly significant. These significant differences strengthen the evidence that there is a significant second-degree curve relationship between maturity and fiber length as shown by the close fitting seen in figure 2.

[5] FISHER, RONALD AYLMER. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 3, rev. and enl., 283 pp., illus. Edinburgh and London. 1930.

The curve respresenting the arithmetic average maturity of the different length classes and the regression curve from the same data are closely related.

The wide variations in soil moisture, coupled with the resultant differences in plant types prevailing in the fields from which the cotton samples were taken, add greatly to the conclusiveness of the results. These fields were not under observation, except on the day of sampling; hence, available data do not permit correlation between seasonal environmental plant growth influences and fiber maturity.

CONCLUSIONS

The longest and also the shortest fibers, in a given lot of cotton, are less mature than the fibers of intermediate lengths.

Fibers toward the top of the range in intermediate lengths are slightly better in maturity than those toward the bottom. Cotton breeders who select progenies with greater proportions of the longer intermediate fibers, unconsciously or otherwise, select toward improvement in fiber maturity.

A MOSAIC DISEASE OF TURNIP [1]

By C. M. TOMPKINS

Assistant plant pathologist, California Agricultural Experiment Station [2]

INTRODUCTION

A mosaic disease of turnip (*Brassica rapa* L.) occurs on Long Island, N. Y. Typical specimens of this disease were obtained for comparative greenhouse studies with virus diseases affecting certain cultivated crucifers in California (*15*, *16*, *17*, *18*).[3]

It is the purpose of this paper to describe briefly the symptoms of this mosaic disease of turnip from New York, to present experimental evidence on transmission, host range, and properties of the virus, and to discuss its possible relationship to other crucifer viruses.

REVIEW OF LITERATURE

In reviewing the literature, it is apparent in some instances that the names, "turnip mosaic" and "rutabaga mosaic," have been used synonymously. It has seemed advisable, therefore, to include in this review all references pertaining to rutabaga mosaic, as well as to turnip mosaic, irrespective of the identity of the particular virus involved.

In 1921, Gardner and Kendrick (*4*) described a mosaic disease of turnip that was found in Indiana. The causal virus was transmitted to healthy turnip seedlings by rubbing wounded plant parts with cotton soaked in extracted juice from diseased plants. The incubation period ranged from 16 to 26 days. Unsuccessful attempts were made to infect red and white varieties of radish (*Raphanus sativus* L.).

What appears to have been the same disease on turnip, Chinese cabbage (*Brassica pe-tsai* Bailey), and mustard (*B. japonica* Coss) was also described by Schultz (*12*). By means of the leaf-mutilation, rubbing method of inoculation, healthy turnip, Chinese cabbage, and mustard seedlings were infected with extracted juice from the corresponding diseased plants, in 20 to 30 days. Further, when the green peach aphid (*Myzus persicae* (Sulzer)) was transferred to healthy turnip and mustard seedlings after having fed on diseased turnip, Chinese cabbage, and mustard plants, mosaic mottling developed in 12 to 20 days. Evidence for seed transmission was negative, since mustard seed from mosaic plants yielded healthy seedlings.

Gram (*5*) found a mosaic disease of turnip in eight localities in Denmark in 1921. Other plants susceptible to natural infection included swedes or rutabaga (*Brassica campestris* L. var. *napo-brassica* DC.), radish, and charlock (*Sinapsis arvensis* L.).

In a brief report by Thatcher (*14*), reference is made to inoculation experiments which showed that mustard, rutabaga, flat turnip, and

[1] Received for publication February 16, 1938; issued November 1938.

[2] The writer is indebted to Dr. M. W. Gardner for advice and assistance and to Prof. B. A. Madson and W. W. Mackie for providing greenhouse space and facilities. Valuable assistance in the greenhouse work was rendered by employees of the Federal Works Progress Administration.

[3] Italic numbers in parentheses refer to Literature Cited, p. 601.

Chinese cabbage are very susceptible to mosaic. Rape was considered to be less susceptible. The identity of this crucifer virus was not disclosed.

Apparently the preceding citation by Thatcher served merely as a preliminary announcement of a more detailed report on studies of a mosaic disease of crucifers conducted by Clayton (*2*). A mosaic disease of rutabaga was found to be common but not serious on Long Island, N. Y. It was also observed on Brussels sprouts (*Brassica oleracea* L. var. *gemmifera* DC.), cauliflower (*B. oleracea* L. var. *botrytis* L.), and black mustard (*B. nigra* Koch), but not on white turnip. Infection experiments involved the use of the leaf mutilation, rubbing method of inoculation and of the cabbage aphid (*Brevicoryne brassicae* (L.)) and the green peach aphid. The cabbage aphid proved very effective as a vector, but the green peach aphid gave negative results. An incubation period of 3 to 5 weeks was reported. Symptoms of the disease were favored by relatively high temperatures (70° to 80° F.), while lower temperatures (55° to 65°) induced masking. White mustard (*B. alba* Rabenh.), black mustard, Chinese cabbage, turnip, rutabaga, and rape (*B. napus* L.) were susceptible to artificial infection. Brussels sprouts and cauliflower were difficult to infect. Although cabbage (*B. oleracea* L. var. *capitata* L.) was considered to be highly resistant or immune to the disease, the suggestion was advanced that it might act as a carrier. No evidence of seed transmission was obtained. Apparently Clayton did not recognize any differences between the rutabaga mosaic virus with which he worked and the turnip mosaic virus reported elsewhere (*4, 5, 12*).

A mosaic disease of Chinese cabbage, turnip, and mustard occasionally caused 30 percent loss near Fukuoka, Japan, according to Takimoto (*13*). The virus was transmitted by mechanical inoculation to cauliflower but not to cabbage or radish. Aphids (species not given) were held responsible for natural transmission of the disease. Takimoto considered the disease to be similar to those described by Gardner and Kendrick (*4*), Schultz (*12*), and Clayton (*2*).

Samuel (*11*) reported on turnip mosaic in Australia in 1931. A mosaic disease of turnip and rutabaga was reported from Florida by Weber (*19*) in 1932 and from Japan by Hino (*6*) in 1933.

Edson, Miller, and Wood [4] recorded the occurrence of rutabaga mosaic in Connecticut and turnip mosaic in Mississippi in 1934.

A virus disease of cabbage, mustard, turnip, and horseradish (*Radicula armoracia* Robins) was described by Hoggan and Johnson (*7*) in 1935. The virus was transmitted by mechanical inoculation and by the cabbage and green peach aphids to cabbage, black mustard, turnip, tobacco (*Nicotiana tabacum* L.) var. Connecticut Havana No. 38, *N. glutinosa* L., the hybrid *N. tabacum N. glutinosa*, currant tomato (*Lycopersicum pimpinellifolium* Dunal), and spinach (*Spinacia oleracea* L.) var. Bloomsdale. The temperature range favorable to infection was 70° to 80° F. Properties of the virus were given: Thermal death point, 54° C. for a 10-minute exposure; longevity in vitro, less than 3 days at 20°–22°; tolerance to dilution, 1 to 1,000.

[4] EDSON, H. A., MILLER, PAUL R., and WOOD, JESSIE I. DISEASES OF PLANTS IN THE UNITED STATES IN 1934. U. S. Bur. Plant Indus., Plant Disease Reptr. Sup. 90: 75. 1935.

Losses caused by turnip mosaic in Germany, according to Pape (*9*), may vary from 1 to 90 percent. Some varieties of turnip were found to be much more susceptible to infection than others. He reported transmission of the virus to healthy turnip plants by mechanical inoculation and by means of the tarnished plant bug (*Lygus pratensis* (L.)).

Chamberlain (*1*) recognized turnip mosaic on rape plants at Palmerston North, New Zealand, in 1932 and subsequently on rutabagas, rape, and turnips in the same and other districts. He obtained mechanical transmission of the disease by the rubbing method and by means of the cabbage and green peach aphids which commonly occur on cruciferous crops in New Zealand, but failed to give the time required in either case for symptom expression. Tests for seed transmission involved planting seeds derived from mosaic-infected rutabaga plants. Of 432 seedlings, none showed symptoms of mosaic. In host-range studies, no infection, by means of artificial inoculation, was secured on cabbage, cauliflower, Brussels sprouts, broccoli (*Brassica oleracea* L. var. *botrytis* L.), and radish. However, all of these crucifers, except radish, were infected when the green peach aphid served as the vector. Brown necrotic lesions were obtained on tobacco (variety not specified). Annual stock (*Matthiola incana* R. Br. var. *annua* Voss) is a natural host for the turnip mosaic virus which induces flower breaking as well as leaf mottling.[5] Other natural hosts include wallflower (*Cheiranthus cheiri* L.) and a number of common cruciferous weeds. In tests for varietal resistance, one variety of turnip proved highly resistant and several others less so. Recommendations for control of the disease in seed crops were dipping the leaves of plants at transplanting time in a nicotine solution in order to kill the insect vectors, rogueing of all infected plants, avoidance of other cruciferous crops and volunteer seedlings, and, when mosaic appears, spraying the plants with nicotine solution.

Edson and Wood[6] listed turnip mosaic as prevalent in Connecticut in 1935.

In 1936, Kaufmann (*8*) described a virus disease of winter-sown rape (*Brassica rapa* L. var. *oleifera* Delile), rutabaga, and colza (*B. napus* L. var. *oleifera* Delile). Infection of healthy rape, rutabaga, and colza plants resulted from juice inoculations and when the tarnished plant bug was used, both in the greenhouse and in the field. Significant losses were observed only on rutabaga. The identity of this virus was apparently not clearly established.

SYMPTOMS OF THE DISEASE

Studies of the symptoms caused by the turnip mosaic virus from New York have been limited to greenhouse observations. Initial symptoms of the disease on leaves of Purple Top White Globe turnip seedlings consist of a conspicuous, coarse, systemic clearing of the veins, with interveinal mottling, which collectively impart a yellowish caste. The leaves show marked crinkling and slight dwarfing (fig.

[5] Letter dated July 2, 1937, from E. E. Chamberlain, Plant Diseases Division, Plant Research Bureau, Palmerston North, New Zealand.

[6] EDSON, H. A., and WOOD, JESSIE I. DISEASES OF PLANTS IN THE UNITED STATES IN 1935. U. S. Bur. Plant Indus., Plant Disease Reptr. Sup. 96: 200. 1936.

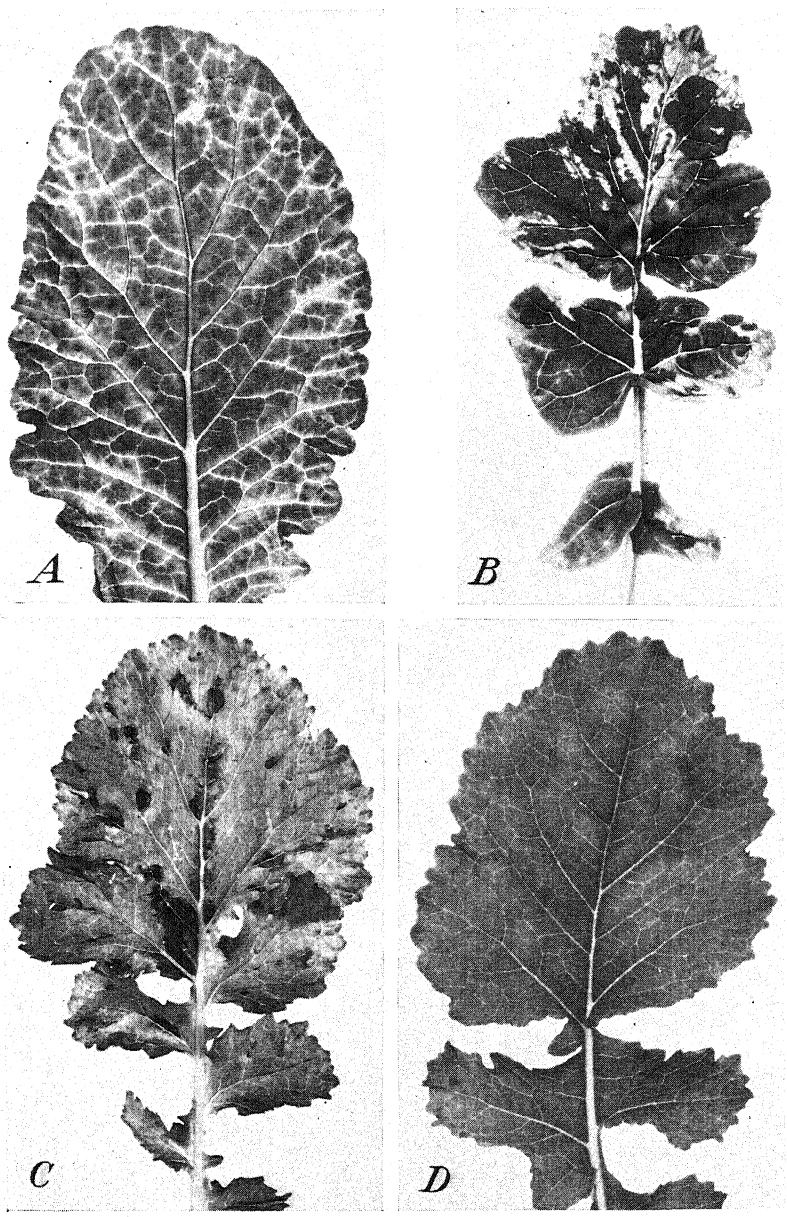


FIGURE 1.—Symptoms produced by the turnip mosaic virus on Purple Top White Globe turnip leaves after artificial inoculation in the greenhouse at 13°–19° C.: *A*, Early symptoms consist of coarse, yellow vein clearing and mottling; *B*, intermediate symptoms consisting of dark-green tissue with numerous raised islands of irregular shape and a small amount of chlorotic tissue; *C*, late symptoms in which the leaf has become very chlorotic with only a few dark-green, raised islands; *D*, noninoculated control.

1, *A*), when contrasted with normal, healthy specimens. Stunting of the plants is also apparent in the early stages of infection. Gradually, as the leaves expand, the coarse, yellow appearance is replaced by a preponderance of very dark-green, irregular, raised islands, interspersed with a restricted amount of chlorotic tissue (fig. 1, *B*). In later stages of infection, the dark-green islands are almost entirely replaced by chlorotic, light-green areas (fig. 1, *C*). Crinkling of the leaves is accentuated in the latter two stages of infection (fig. 1, *B*, *C*) in contrast to the normal, healthy leaf (fig. 1, *D*). Occasionally in some infected plants the leaves were unsymmeterical. Although the turnip mosaic virus causes severe stunting of turnip plants, no lethal effect has been observed on this or any other susceptible host.

MATERIALS AND METHODS

In 1934, 12 white turnip stecklings showing pronounced symptoms of mosaic infection were collected in a commercial planting near Mattituck, Long Island, N. Y., and forwarded to Berkeley, Calif. Within several weeks after transplanting, these stecklings produced new leaves, all of which were mottled. Transfers were then made to healthy Purple Top White Globe turnip seedlings by mechanical inoculation. The virulence of, and the symptoms produced by, this virus have remained unchanged through successive monthly transfers since 1934. Purple Top White Globe turnip seedlings were used as the standard test plant for recovery of the virus from infected plants and for property studies.

All inoculations were made in a greenhouse where temperatures ranged from 13° to 19° C. The methods followed were essentially those described in recent papers (*16*, *18*). Mechanical inoculations were made by dusting the leaves with 600-mesh, powdered carborundum (*10*) and lightly rubbing with absorbent cotton dipped in juice from a diseased plant.

EXPERIMENTAL RESULTS

TRANSMISSION

The turnip mosaic virus can be transferred to healthy turnip seedlings by mechanical inoculation with or without carborundum (*10*), but the percentage of infection is reduced approximately 50 percent when this abrasive is omitted. As a result of numerous tests, the incubation period was determined as ranging from 13 to 21 days.

Clayton (*2*) used both the cabbage aphid and the green peach aphid in his insect transmission studies with the rutabaga mosaic virus, finding that the latter was much less efficient. Since Essig (*3*) found that the cabbage and green peach aphids breed naturally on cultivated and wild crucifers in California and other Western States and because the New York turnip mosaic virus may eventually be found in California, it seemed desirable to test the two aphid species under greenhouse conditions.

Following the procedure outlined in a recent paper (*18*), noninfective cabbage and green peach aphids[7] were fed on recently infected

[7] Noninfective cabbage and green peach aphids, previously identified as such by E. O. Essig, were kindly supplied by H. H. P. Severin and J. H. Freitag, Division of Entomology and Parasitology, University of California.

turnip plants for 24 to 48 hours, after which they were transferred in lots of approximately 20 aphids each to healthy turnip seedings. After 24 hours, all plants were sprayed with nicotine sulphate solution. Healthy turnip seedlings infested with noninfective aphids and noninoculated plants free from aphids served as controls.

Of 20 turnip plants infested with viruliferous cabbage aphids, 16 showed typical symptoms of the disease in 15 to 17 days, while 4 plants damped off. When infective green peach aphids were placed on 20 turnip plants, 100-percent infection occurred, the incubation period ranging from 12 to 18 days. The virus was recovered from all infected plants by mechanical inoculation. None of the controls became diseased. Other possible means of transmission were not investigated.

EXPERIMENTAL HOST RANGE

Young seedlings of various turnip and rutabaga varieties were tested by mechanical inoculation, with a suitable number of plants for controls. The following varieties of turnip proved to be highly susceptible: Amber or Yellow Globe, Bortfelder, Cowhorn, Early Purple Top Milan, Early White Flat Dutch, Extra Early White Milan, Large White Norfolk, Orange Jelly or Golden Ball, Purple Strap Leaf, Purple Top Strap Leaved Early, Purple Top Yellow Aberdeen, Seven Top, Shogoin or Japanese, Snowball, Southern Prize, White Egg, and White Milan. Of rutabaga varieties, the following varieties proved to be highly susceptible: American Purple Top, Hartleys Bronze Top, Large White, and Monarch or Tankard. The variety Long Island Improved was only slightly susceptible to infection.

By means of mechanical inoculation, the turnip mosaic virus from New York was transmitted to 18 species of plants representing 12 genera in 6 families, as follows:

Cruciferae:
- Cabbage (*Brassica oleracea* L. var. *capitata* L.).
- Cauliflower (*B. oleracea* var. *botrytis* L.).
- Rutabaga (*B. campestris* L. var. *napo-brassica* DC.).
- Turnip (*B. rapa* L.).
- Leaf or Chinese mustard (*B. juncea* Coss).
- Chinese cabbage (*B. pe-tsai* Bailey).
- Wild yellow mustard (*B. campestris* L.).
- *B. adpressa* Boiss.
- Shepherds-purse (*Capsella bursa-pastoris* Medic.).
- Annual stock (*Matthiola incana* R. Br. var. *annua* Voss).
- Dames violet (*Hesperis matronalis* L.).
- Virginian stock (*Malcomia maritima* R. Br.).
- Honesty (*Lunaria annua* L.).
- Chinese radish (*Raphanus sativus* L. var. *longipinnatus* Bailey).

Chenopodiaceae: Lambsquarters or white pigweed (*Chenopodium album* L.)
Resedaceae: Mignonette (*Reseda odorata* L.).
Begoniaceae: Fibrous-rooted begonia (*Begonia semperflorens* Link and Otto).
Verbenaceae: Garden verbena (*Verbena hybrida* Voss).
Solanaceae:
- Turkish tobacco (*Nicotiana tabacum* L.).
- *N. glutinosa* L.
- Petunia (*Petunia hybrida* Hort.).

In the family Cruciferae, all susceptible plants showed systemic infection. These include Winter Colma cabbage (fig. 2) with numerous chlorotic lesions which later became necrotic around the edges; February, April, Danish Perfection, Dryweather Danish Giant,

Early March, Early Snowball, and Super Snowball cauliflower with chlorotic to yellow lesions; Long Island Improved rutabaga with chlorotic lesions; Flat Turnip and Florida Broadleaf varieties of leaf or Chinese mustard with leaf mottling and rugosity; pe-tsai with coarse yellow vein banding and leaf distortion; *Brassica adpressa*, wild yellow mustard, and shepherds-purse with coarse mottling; Fiery Blood Red annual stock with coarse vein clearing, diffuse mottling, and occasional midrib distortion of leaves and flower breaking; dames violet with a fine type of mottling followed by small

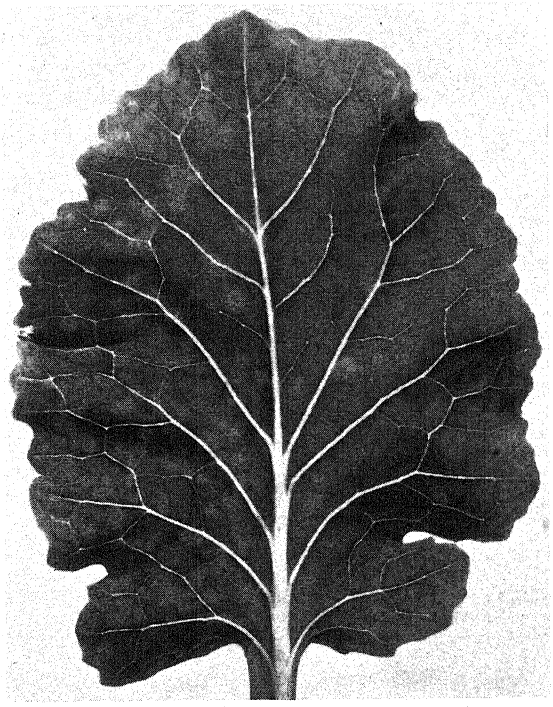


FIGURE 2.—Early symptoms, consisting of small, diffuse, chlorotic rings, on Winter Colma cabbage leaf 27 days after artificial inoculation with the turnip mosaic virus in the greenhouse at 13° to 19° C.

necrotic lesions, raised dark-green islands, and marked bleaching; and Virginian stock, honesty, and Chinese radish with mottling.

Infection of lambsquarters or white pigweed was indicated by local, yellow lesions on inoculated leaves only. Later, these became necrotic, with some coalescence. Pronounced mottling of leaves characterized infection of mignonette, garden verbena, and petunia. Small chlorotic lesions appeared on leaves of infected fibrous-rooted begonia. Local chlorotic lesions which later became necrotic occurred only on inoculated leaves of Turkish tobacco and *Nicotiana glutinosa* (fig. 3). The virus was recovered from all infected plants except from lambsquarters, mignonette, and fibrous-rooted begonia.

No infection was obtained by mechanical inoculation in 53 species of plants representing 43 genera in 23 families, as follows:

Cruciferae:
Kale (*Brassica oleracea* L. var. *acephala* DC.).
Brussels sprouts (*B. oleracea* var. *gemmifera* DC.).
Sprouting broccoli (*B. oleracea* var. *botrytis* L.).
Kohlrabi (*B. oleracea* var. *caulorapa* DC.).
Rape (*B. napus* L.).
Black mustard (*B. nigra* Koch).
White mustard (*B. alba* Rabenh.).
Charlock (*B. arvensis* (L.) Ktze.).
B. integrifolia O. E. Schulz var. *chevalieri* R. Porteres.
Wallflower (*Cheiranthus cheiri* L.).
Evening scented stock (*Matthiola bicornis* DC.).

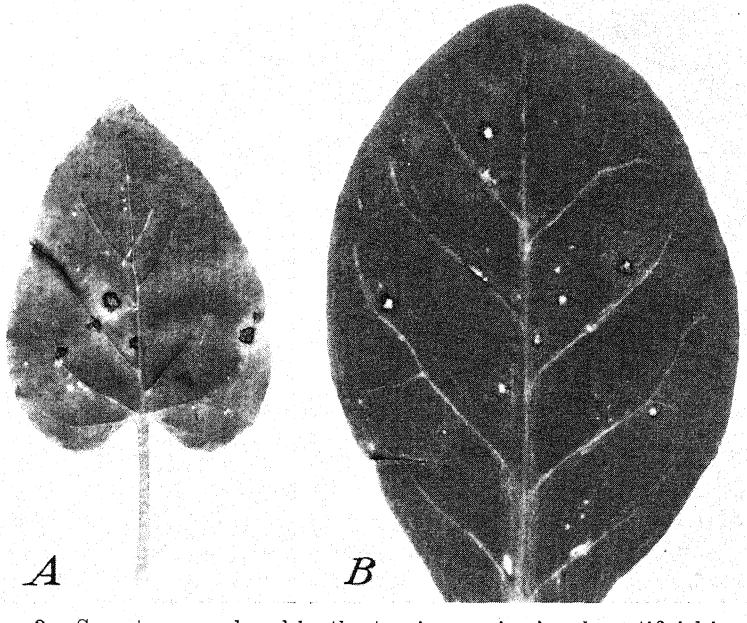


FIGURE 3.—Symptoms produced by the turnip mosaic virus by artificial inoculation in the greenhouse at 13° to 19° C.: *A*, Local chlorotic lesions on inoculated leaf of *Nicotiana glutinosa* which later became necrotic with tan centers; *B*, local necrotic lesions on leaf of *N. tabacum* var. Turkish.

Sweet alyssum (*Alyssum maritimum* Lam.).
Radish (*Raphanus sativus* L.) var. White Icicle and Crimson Giant Forcing.
Gramineae: Corn (*Zea mays* L.) var. Golden Bantam.
Polygonaceae: Rhubarb (*Rheum rhaponticum* L.).
Chenopodiaceae:
Sowbane or nettleleaf goosefoot (*Chenopodium murale* L.).
Spinach (*Spinacea oleracea* L.) var. Bloomsdale.
Caryophyllaceae:
Sweet-william (*Dianthus barbatus* L.).
Babysbreath (*Gypsophila panicualta* L.).
Ranunculaceae: Rocket larkspur (*Delphinium ajacis* L.).
Rosaceae: *Geum chiloense* Balb.
Leguminosae:
Garden pea (*Pisum sativum* L.) var. Alderman.
Broadbean (*Vicia faba* L.).

Tropaeolaceae: Garden nasturtium (*Tropaeolum majus* L.).
Euphorbiaceae: Castor-bean (*Ricinus communis* L.).
Violaceae: Pansy (*Viola tricolor* L.).
Onagraceae:
Clarkia elegans Dougl.
Godetia grandiflora Lindl.
Umbelliferae: Celery (*Apium graveolens* L.) var. Golden Self Blanching.
Plumbaginaceae: Everlasting or sea-lavender (*Statice latifolium* Kuntze).
Boraginaceae:
Forget-me-not (*Myosotis alpestris* Schmidt).
Common heliotrope (*Heliotropium peruvianum* L.).
Labiatae: Flowering sage (*Salvia farinacea* Benth.).
Solanaceae:
Solanum aviculare Forst.
Potato (*S. tuberosum* L.).
Tomato (*Lycopersicum esculentum* Mill. var. *vulgare* Bailey) var. Early Santa Clara Canner.
Currant tomato (*L. pimpinellifolium* Dunal).
Nicotiana langsdorfii Weinm.
N. rustica L. var. *humulis* Schrank.
Tobacco (*N. tabacum* L.) var. White Burley.
Jimsonweed (*Datura stramonium* L.).
Scrophulariaceae:
Snapdragon (*Antirrhinum majus* L.).
Penstemon or beardtongue (*Penstemon barbatus* Nutt.).
Dipsaceae: Mourning bride or pincushion flower (*Scabiosa atropurpurea*.).
Cucurbitaceae: Cucumber (*Cucumis sativus* L.).
Campanulaceae: Canterbury-bells (*Campanula medium* L.).
Lobeliaceae: *Lobelia hybrida* Hort.
Compositae:
Head lettuce (*Lactuca sativa* L. var. *capitata* Hort.) var. New York and Tom Thumb.
Dandelion(*Taraxacum officinale* Weber).
Annual marguerite (*Chrysanthemum coronarium* L.).
English daisy (*Bellis perennis* L.).
African marigold (*Tagetes erecta* L.).
French marigold (*T. patula* L.).
Winter Cape-marigold (*Dimorphotheca aurantiaca* DC.).
Gaillardia pulchella Foug. var. *picta* Gray.
Cineraria (*Senecio cruentus* DC.).

Subsequent inoculations to turnip with extracted juice from inoculated plants of the above-mentioned plant species failed to cause infection.

PROPERTIES OF THE VIRUS

Determinations were made on longevity in vitro, inactivation temperature, and tolerance to dilution, and the results are given in table 1.

Virus samples consisted of 2 cc of undiluted juice, from recently infected turnip plants, in small, thin-walled test tubes. In determining resistance to aging in vitro, samples were stored at a constant temperature of 22° C. Resistance to heating was determined by heating virus samples in a thermostatically controlled water bath for 10 minutes. Virus dilutions were made with distilled water. Immediately following a specific treatment, young Purple Top White Globe turnip plants were then tested for infectivity by mechanical inoculation.

The turnip mosaic virus was found to be infective for 48 hours after aging in vitro, but was inactivated after 72 hours. The virus was infectious after heating for 10 minutes at various temperatures not exceeding 60° C., but was inactivated at 63° C. A dilution tolerance

of 1:3,000 was established. In each of the three tests, 25 noninoculated turnip plants served as controls and they continued healthy throughout.

TABLE 1.—*Longevity in vitro, inactivation temperature, and tolerance to dilution of the turnip mosaic virus*

[Five trials on 25 plants in all cases]

LONGEVITY IN VITRO, 22° C.

Age (hours)	Plants infected	Age (hours)	Plants infected
	Number		*Number*
0	25	96	0
24	13	120	0
48	2	144	0
72	0	168	0

INACTIVATION TEMPERATURE (10 MINUTES)

Temperature (°C.)	Plants infected	Temperature (°C.)	Plants infected
	Number		*Number*
50	17	65	0
55	10	70	0
60	2	(1)	21
63	0		

TOLERANCE TO DILUTION

Dilution	Plants infected	Dilution	Plants infected
	Number		*Number*
0	25	1:2,000	4
1:10	20	1:3,000	4
1:100	17	1:4,000	0
1:500	7	1:5,000	0
1:1,000	8		

[1] Not treated.

DESCRIPTION OF THE TURNIP MOSAIC VIRUS FROM NEW YORK

Transmitted in greenhouse tests by means of the cabbage aphid (*Brevicoryne brassicae*) and the green peach aphid (*Myzus persicae*). Transmissible by mechanical inoculation, using expressed juice with or without powdered carborundum. Incubation period 13 to 21 days when Purple Top White Globe turnips were used. Resistance to aging in vitro between 2 and 3 days. Inactivation temperature between 60° and 63° C. for 10-minute exposure. Tolerance to dilution approximately 1 to 3,000. White turnip (*Brassica rapa* L.) and certain other vegetable and ornamental crucifers are susceptible. On turnip, early symptoms consist of diffuse mottling of the leaves; later, irregular-shaped, raised dark-green islands are scattered on a markedly chlorotic background. Slight distortion and rugosity of leaves in late stages of infection. Local necrotic lesions produced on *Nicotiana glutinosa* and *N. tabacum*. Chinese radish susceptible, but not ordinary radish.

COMPARISON OF THE TURNIP MOSAIC VIRUS FROM NEW YORK WITH CERTAIN OTHER CRUCIFER VIRUSES

In a recent paper (*16*) the writer briefly compared the symptoms produced on certain cultivated crucifers by the cauliflower mosaic and turnip mosaic (from New York) viruses. When sprouting

broccoli, cabbage, cauliflower, kohlrabi, radish, and turnip seedlings were inoculated with the cauliflower mosaic virus, early symptoms were manifested by vein clearing (*16, fig. 3, C*; fig. 1, *A*) (fig. 4). The turnip mosaic virus, however, produced different symptoms on infected plants: Cabbage, chlorotic rings later becoming necrotic; cauliflower, pale-green to yellow lesions (*16, fig. 5, A, B*). As shown in this paper, a coarse leaf mottle characterizes infection of turnip. Sprouting broccoli, kohlrabi, and radish were not infected by the turnip mosaic virus. On annual stock, the cauliflower mosaic virus caused a coarse vein clearing of the leaves (*16, fig. 4, A, B, C*) but not flower breaking; the turnip mosaic virus induced not only coarse vein clear-

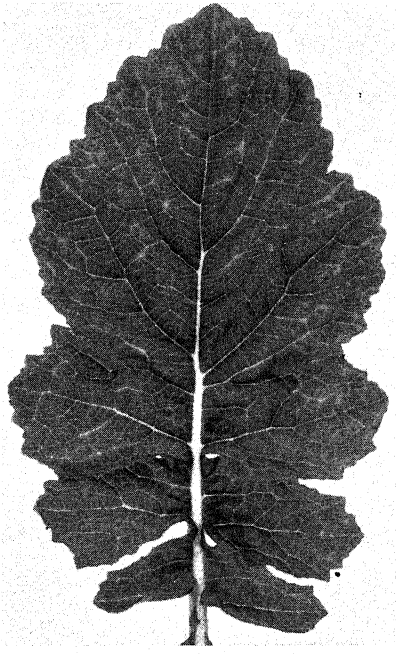


FIGURE 4.—Vein-clearing symptoms produced by the cauliflower mosaic virus on Purple Top White Globe turnip leaf by artificial inoculation in the greenhouse at 13° to 19° C.

ing and diffuse mottling but also flower breaking. No infection was obtained with the cauliflower mosaic virus on *Nicotiana glutinosa* and Turkish tobacco (*16*), whereas the turnip mosaic virus caused necrotic lesions to form on inoculated leaves of both *Nicotiana* species. These viruses can also be further differentiated by comparing their properties.

Recently, Tompkins and Thomas (*18*) have described a mosaic disease of Chinese cabbage in which it was shown that this virus can readily be differentiated from the turnip mosaic and cauliflower mosaic viruses on such hosts as cabbage, Chinese cabbage, cauliflower, and turnip.

It is believed the evidence submitted in the present paper is sufficient to differentiate the turnip mosaic virus from other viruses recently described (*16, 17, 18*).

DISCUSSION

A review of the literature on turnip mosaic (*1, 4, 5, 6, 7, 9, 11, 12, 13, 19*) and rutabaga mosaic (*2, 6, 8, 14, 19*) indicates a lack of agreement on the part of the workers concerned, specific examples of which may be cited. Although it seems to be generally agreed that the principal symptom of the disease on turnip consists of leaf mottling, additional symptoms unlike in any two instances are given by several writers (*1, 2, 4, 5, 7, 9, 12*). All but Gram (*5*) reported infection by mechanical means. Concerning insect transmission, Schultz (*12*) proved the green peach aphid was a vector; Pape (*9*) considered the tarnished plant bug was responsible for natural spread of the disease in Germany; while Hoggan and Johnson (*7*) and Chamberlain (*1*) found that both the green peach and cabbage aphids were efficient vectors. In studies on the rutabaga mosaic virus, Clayton (*2*) stated the green peach aphid gave unsatisfactory results, but transmission occurred by means of the cabbage aphid. Schultz (*12*), Clayton (*2*), and Chamberlain (*1*) reported negative results in tests for seed transmission but no data were given by others. According to Gardner and Kendrick (*4*), the incubation period for artificial infection ranged from 16 to 26 days while Clayton (*2*) records 3 to 5 weeks. Schultz (*12*) reported a period of 12 to 20 days for the green peach aphid.

Relative to host range, Gardner and Kendrick (*4*) and Chamberlain (*1*) were unable to infect radish. Unfortunately, other workers failed to test this plant for susceptibility. Conversely, Gram (*5*) observed infection of radish. Infection of rutabaga was reported only by Gram (*5*), Clayton (*2*), and Chamberlain (*1*); the description of symptoms differs in the latter two instances. There is no close agreement concerning symptoms on such hosts as Brussels sprouts and cauliflower based on the studies of Clayton (*2*) and Chamberlain (*1*). Clayton (*2*) found that cabbage was practically immune, Hoggan and Johnson (*7*) observed chlorotic rings, and Chamberlain (*1*) noticed only a faint mottle on the leaves. The writer (*16*) has previously reported light-green to yellow lesions which become necrotic around the edges with age. Spinach and currant tomato were reported as hosts by Hoggan and Johnson (*7*), but the writer has failed to obtain confirmatory results. To date, data on the properties of a turnip mosaic virus have been published only by Hoggan and Johnson (*7*). They used tobacco as a test host and since the writer used turnip a satisfactory comparison cannot be made.

Inevitably these differences have led to such questions as: Was the same turnip mosaic virus, or the same rutabaga mosaic virus, under the consideration by the different workers; are the turnip mosaic and rutabaga mosaic viruses identical? It is believed satisfactory answers to these questions cannot be given on the basis of available information and that any definite conclusions would not be justified.

SUMMARY

A mosaic disease of turnip, prevalent on Long Island, N. Y., is described.

Turnip mosaic is characterized by coarse vein clearing of the leaves in early stages of infection followed by conspicuous mottling with raised islands, crinkling, and stunting of the plants.

Under greenhouse conditions the turnip mosaic virus was readily transmitted by *Myzus persicae* and *Brevicoryne brassicae*. The virus was also transmitted by mechanical inoculation using carborundum as an abrasive; the incubation period ranged from 13 to 21 days. In longevity tests, the virus was active at the end of 2 days but inactivated after aging for 3 days at 22° C. Its inactivation temperature lies between 60° and 63°. A tolerance to dilution of 1 to 3,000 was established.

The host range includes 18 species of plants representing 12 genera in 6 families; 11 species belong to the family Cruciferae and include cabbage, cauliflower, rutabaga, leaf or Chinese mustard, pe-tsai, annual stock, dames violet, Virginian stock, honesty, and Chinese radish.

LITERATURE CITED

(1) CHAMBERLAIN, E. E.
1936. TURNIP-MOSAIC. A VIRUS DISEASE OF CRUCIFERS. New Zeal. Jour. Agr. 53: 321–330, illus.

(2) CLAYTON, E. E.
1930. A STUDY OF THE MOSAIC DISEASE OF CRUCIFERS. Jour. Agr. Research 40: 263–270, illus.

(3) ESSIG, E. O.
1929. INSECTS OF WESTERN NORTH AMERICA. . . 1035 pp., illus. New York.

(4) GARDNER, MAX W., and KENDRICK, JAMES B.
1921. TURNIP MOSAIC. Jour. Agr. Research 22: 123–124, illus.

(5) GRAM, E.
1925. MOSAIKSYGE HOS KORSBLOMSTREDE. [MOSAIC IN CRUCIFERS.] Dansk Frøavl. 8: 41–42. [Abstract in Bot. Abs. 15: 782. 1926.]

(6) HINO, IWAO.
1933. LIST OF PLANTS SUSCEPTIBLE TO MOSAIC AND MOSAIC-LIKE DISEASES. Miyazaki Col. Agr. and Forestry Bull. 5: 97–110.

(7) HOGGAN, ISMÉ A., and JOHNSON, JAMES.
1935. A VIRUS OF CRUCIFERS AND OTHER HOSTS. Phytopathology 25: 640–644, illus.

(8) KAUFMANN, O.
1936. EINE GEFÄHRLICHE VIRUSKRANKHEIT AN RÜBSEN, RAPS UND KOHLRÜBEN. Arb. Biol. Reichsanst. Land u. Forstw. 21: [605]–623, illus.

(9) PAPE, HEINRICH.
1935. ÜBER EINE MOSAIKKRANKHEIT DER KOHLRÜBE. Deut. Landw. Presse 62: 319–320, illus.

(10) RAWLINS, T. E., and TOMPKINS, C. M.
1936. STUDIES ON THE EFFECT OF CARBORUNDUM AS AN ABRASIVE IN PLANT VIRUS INOCULATIONS. Phytopathology 26: 578–487, illus.

(11) SAMUEL, GEOFFREY.
1931. SUMMARY OF PLANT DISEASE RECORDS IN SOUTH AUSTRALIA FOR THE TWO YEARS ENDING JUNE 30, 1930. So. Aust. Dept. Agr. Jour. 34: 746.

(12) SCHULTZ, E. S.
1921. A TRANSMISSIBLE MOSAIC DISEASE OF CHINESE CABBAGE, MUSTARD, AND TURNIP. Jour. Agr. Research 22: 173–178, illus.

(13) TAKIMOTO, S.
1930. [ON THE MOSAIC DISEASE OF CHINESE CABBAGE AND OTHER CRUCIFERS.] Nippon Engei Zasshi 42: 5–7. [In Japanese.]

(14) THATCHER, R. S.
1927. DISEASES OF CRUCIFEROUS CROPS ON LONG ISLAND. N. Y. State Agr. Expt. Sta. Ann. Rept. (1925–26) 45: 29–30.

(15) TOMPKINS, C. M.
1934. BREAKING IN STOCK (MATHIOLA INCANA), A VIROSIS. (Abstract) Phytopathology 24: 1137.
(16) ———
1937. A TRANSMISSIBLE MOSAIC DISEASE OF CAULIFLOWER. Jour. Agr. Research 55: 33–46, illus.
(17) ——— GARDNER, M. W., and THOMAS, H. REX.
1937. BLACK RING, A VIROSIS OF CABBAGE AND OTHER CRUCIFERS. (Abstract) Phytopathology 27: 955–956.
(18) ——— and THOMAS, H. REX.
1938. A MOSAIC DISEASE OF CHINESE CABBAGE. Jour. Agr. Research 56: 541–551, illus.
(19) WEBER, GEORGE F.
1932. SOME DISEASES OF CABBAGE AND OTHER CRUCIFERS IN FLORIDA. Fla. Agr. Expt. Sta. Bull. 256, 62 pp., illus.

ASSAYS OF RIBOFLAVIN AND THE FILTRATE FACTOR IN CERTAIN MILK PRODUCTS [1]

By T. H. JUKES, *junior poultry husbandman*, and G. A. RICHARDSON, *assistant dairy chemist, California Agricultural Experiment Station* [2]

INTRODUCTION

Two factors in the vitamin G group are known to be needed by chicks.[3] The first of these is riboflavin (flavin, lactoflavin), which is of great importance for growth and hatchability, and the second is the "filtrate factor," which prevents a dermatitis caused by a dietary deficiency in chicks. Dried milk and dried whey supply both of these vitamins (*4*). Milk products are particularly important in poultry rations as sources of riboflavin, since many feeding stuffs, such as the cereal grains, are deficient in this substance. Heiman and Carver (*1*) have recently found that a sample of neutralized dried whey contained between 10 and 20 percent more vitamin G, and a sample of dried sweet-cream buttermilk contained between 90 and 100 percent more vitamin G, than a sample of dried skim milk. The samples were prepared from similar supplies of milk collected during 1 week.

In the present investigation two series of spray-dried milk products were studied. Each series originated from a single batch of whole milk. Each product was biologically assayed for the filtrate factor and for riboflavin by means of chick growth.

PREPARATION OF SPRAY-DRIED MILK PRODUCTS

For the products prepared in November 1935, a mixed composite of 7,419 pounds of milk was obtained from 23 patrons. Approximately 1,600 pounds of this was Guernsey milk; the remainder was Jersey milk. The cows received pasture, rye hay, carrots, beets, and millfeed consisting largely of rolled barley, coconut meal, and beet pulp. The milk was standardized down to 4.5 percent of milk fat (Babcock) by the removal of cream. It was then subdivided into appropriate lots for the preparation of the respective products.

For the products prepared in May 1936, a mixed composite of 5,660 pounds of milk from 19 patrons was used. This milk was obtained from about the same territory as the November milk, although not necessarily from the same patrons. Pasture and some millfeed constituted the main ration of the cows. This milk was standardized down to 4.287 percent of fat (Mojonnier) (4.34 percent Babcock) by the removal of cream. It was then subdivided into lots.

The buttermilk was obtained by churning the cream received the day previous in the same plant, although not necessarily from the same patrons who furnished the milk for the remainder of the products.

---

[1] Received for publication September 16, 1937; issued November 1938.

[2] The samples used in this work were obtained through the cooperation of Dr. Paul D. V. Manning and Mr. Henry Pollard of the Western Condensing Co., San Francisco. The Golden State Co., San Francisco, placed their spray-drying plant at Ferndale, Calif., at the disposal of the writers for the preparation of the samples. The analyses of the products were made by Miss M. A. Ashenfelder, analyst, University of California, Davis, Calif. Washed casein was furnished by the California Milk Products Corporation, Gustine, Calif., through the courtesy of J. Chrisman.

[3] Italic numbers in parentheses refer to Literature Cited, p. 609.

All other products in each series were prepared from the one lot of milk.

The whole milk, skim milk, and buttermilk were dried in the usual manner by the Gray-Jensen process. The various casein wheys were obtained by the customary technique. The hydrochloric acid whey was neutralized with soda ash; the sulphuric acid whey, with lime. The lactic acid whey was not neutralized. The rennet whey was the usual Cheddar-cheese whey. Table 1 shows the analyses of the various products.

TABLE 1.—*Chemical analyses of milk and dried-milk products*

Product	Month prepared	Fluid product			Dried product			
		Fat [1]	Total solids	pH	Fat	Total solids	Solids not fat	Ash
		Percent	*Percent*		*Percent*	*Percent*	*Percent*	*Percent*
Whole milk	November	4.5			30.16	94.14	62.94	4.76
	May	4.287	13.12	6.41	32.35	99.01	66.66	4.81
Skim milk	November		10.28			98.32		7.44
	May	.071	9.41	6.44	1.29	97.72	96.43	9.05
Buttermilk	November		9.80			98.32		7.24
	May	.465	9.43	6.36	4.84	98.15	93.31	6.85
Rennet whey (Cheddar)	November		6.65			97.91		6.56
	May	.092	6.27	6.10	1.43	97.62	97.19	6.45
Hydrochloric acid casein whey (neutralized).	November		6.71			97.70		11.56
	May		6.66	6.14	.30	98.79	98.49	11.77
Sulphuric acid casein whey (neutralized).	November		6.92			96.42		14.62
	May		6.54	6.46	.38	97.38	97.00	11.50
Lactic acid casein whey	November		6.62			91.92		10.98
	May		5.95	4.73	.35	97.60	97.25	8.99

[1] Fat was determined by the Mojonnier method, except for the fluid whole milk for November.

BIOLOGICAL ASSAY FOR THE FILTRATE FACTOR

METHOD

The method used to determine the filtrate factor was the one employed in previous investigations (*2*, *6*). Chicks were placed on an ordinary mixed diet at hatching and kept on the diet for 6 to 8 days. They were then placed on a heated diet of natural foodstuffs (*2*, *5*) for 8 to 10 days, after which they were weighed and divided into groups of 10. The test diets were then fed for 12 to 14 days, and the growth response was noted. Each series was controlled by a group on the basal heated diet 80G (*2*) and a group on the positive control diet, which consisted of the heated diet plus 10 percent of rice-bran filtrate (*2*). The filtrate factor content of a sample of dried whey, for example, was computed as follows:

$$\text{Filtrate factor content of whey sample in units per gram} = \frac{\text{Growth on diet supplemented with whey minus growth on basal diet}}{\left\{\begin{matrix}\text{Growth on positive control diet}\\ \text{minus growth on basal diet}\end{matrix}\right\} \times \begin{matrix}\text{Grams of whey in 1}\\ \text{g. of test diet.}\end{matrix}}$$

If, for example, during the assay period the average growth was 59 g on the positive control diet, 4 g on the basal diet, and 33 g on a test diet containing 12 percent of dried whey, the filtrate-factor content of the dried whey would then be computed as

$$\frac{33-4}{(59-4)\ 0.12} = 4.4 \text{ units per gram } (2).$$

The diets were adjusted to keep the nutritive ratio fairly uniform. Table 2 illustrates the composition of the diets in a typical test. The whey adsorbate used was assayed for riboflavin with chicks. From 1.3 to 1.5 percent supplied enough riboflavin for maximal growth between 2 and 4 weeks when fed in conjunction with diet 96.

TABLE 2.—*Composition of diets* [1] *(expressed in parts) used in a typical assay of dried-milk products for the filtrate factor*

Constituent	Composition of diet No. —								
	80G 90	80G 91	80G 92	80G 93	80G 100	80G 96	80G 95	80G 97	80G 98
Yellow corn meal heated at 120° C. for 24 hours	34	46	46	46	46	46	46	56	56
Wheat middlings heated at 120° C. for 24 hours	25	25	25	25	25	25	25	25	25
Commercial casein heated at 120° C. for 24 hours	10	4	10	10	4	10	10	12	12
Sodium chloride	1	1	1	1	1	1	1	1	1
Ground limestone	1	1	1	1	1	1	1	1	1
Steamed bonemeal	1	1	1	1	1	1	1	1	1
Whey adsorbate	2	2	2	2	2	2	2	2	2
Cod-liver oil, medicinal	1	2	2	2	2	2	2	2	2
Dried whole milk	25	------	------	------	------	------	------	------	------
Dried skim milk	------	------	------	------	18	------	------	------	------
Dried buttermilk	------	18	------	------	------	------	------	------	------
Dried rennet whey	------	------	12	------	------	------	------	------	------
Dried hydrochloric acid casein whey	------	------	------	12	------	------	------	------	------
Dried lactic acid casein whey	------	------	------	------	------	12	------	------	------
Sulphuric acid casein whey	------	------	------	------	------	------	12	------	------
Rice-bran filtrate	------	------	------	------	------	------	------	------	10

[1] Hexane extract of alfalfa meal, equivalent to 1 percent of alfalfa meal, evaporated on all diets to supply vitamin K.

RESULTS

The results are summarized in table 3, which compares the various products with respect to their filtrate-factor content in units per gram of the spray-dried material. The dried wheys contained a higher concentration of the filtrate factor because this vitamin is not readily adsorbed and remains in solution in whey after the removal of the fat and casein. Table 3 shows that there may be considerable disparity between duplicate assays; but when average values were taken as in table 4 the products prepared in May arranged themselves in the same order of efficacy as those prepared in November. Differences between a product prepared in November and the corresponding product prepared in May were insignificant.

TABLE 3.—*Filtrate factor content of dried milk and whey samples prepared in November 1935 and May 1936 as determined by biological assay with chicks*

Diet No.	Supplement (dried product)	Proportion of supplement in diet	Length of assay period	Gain on supplemented diet	Gain on basal diet	Gain on positive control	Filtrate factor per gram of supplement
		Percent	*Days*	*Grams*	*Grams*	*Grams*	*Units*
NOVEMBER SAMPLES							
80E 58	Skim milk	18	13	32	4	59	2.8
80E 59	Whole milk	25	13	36	4	59	2.3
80E 60	Buttermilk	18	13	44	4	59	4.0
80E 61	Rennet whey	12	13	43	4	59	5.9
80E 62	Hydrochloric acid casein whey	12	13	30	4	59	3.9
80E 63	Lactic acid casein whey	12	13	34	4	59	4.5
80E 64	Sulphuric acid casein whey	12	13	35	4	59	4.7
80E 68	Skim milk	12	14	25	8	72	2.2
80E 69	Whole milk	12	14	13	8	72	0.7
80E 70	Buttermilk	12	14	27	8	72	2.5
80E 71	Rennet whey	12	14	41	8	72	4.3
80E 72	Hydrochloric acid casein whey	12	14	28	8	72	2.6
80E 73	Lactic acid casein whey	12	14	30	8	72	2.9
80E 74	Sulphuric acid casein whey	12	14	39	8	72	4.0
MAY SAMPLES							
80G 100	Skim milk	18	12	35	6	64	2.8
80G 90	Whole milk	25	12	29	6	64	1.6
80G 91	Buttermilk	18	12	39	6	64	3.2
80G 92	Rennet whey	12	12	41	6	64	5.0
80G 93	Hydrochloric acid casein whey	12	12	26	6	64	2.9
80G 94	Lactic acid casein whey	12	12	40	6	64	4.9
80G 96	Lactic acid casein whey	12	12	38	6	64	4.6
80G 95	Sulphuric acid casein whey	12	12	38	6	64	4.6
80G 110	Skim milk	24	13	25	8	55	1.5
80G 103	Whole milk	33	13	34	8	55	1.7
80G 104	Buttermilk	24	13	46	8	55	3.4
80G 105	Rennet whey	16	13	54	8	55	5.1
80G 106	Hydrochloric acid casein whey	17	13	34	8	55	3.2
80G 108	Lactic acid casein whey	15	13	30	8	55	3.1
80G 107	Sulphuric acid casein whey	17	13	50	8	55	5.3

TABLE 4.—*Average values obtained for filtrate factor content of dried-milk products in units per gram*

[Summarized results of table 3]

Dried-milk product	November 1935 samples	May 1936 samples	Dried-milk product	November 1935 samples	May 1936 samples
	Units	*Units*		*Units*	*Units*
Skim milk	2.5	2.2	Hydrochloric acid casein whey	3.2	3.0
Whole milk	1.5	1.6	Lactic acid casein whey	3.7	4.2
Buttermilk	3.2	3.3	Sulphuric acid casein whey	4.4	5.0
Rennet whey	5.1	5.6			

BIOLOGICAL ASSAY FOR LACTOFLAVIN

METHOD

In an assay for the filtrate factor, the basal diet must contain an excess of riboflavin (*6*). For the same reason, a basal diet for the biological assay of riboflavin must contain an excess of the filtrate factor. Diet 96 (*7*) was used. The treatment of the chicks and the

method of calculating the riboflavin content of the supplements resembled those employed in filtrate-factor assays except, of course, that diet 96 was used instead of diet 80G and the supplement for the positive control diet was whey adsorbate instead of rice-bran filtrate. Table 5 shows the composition of the diets and some of the results obtained. The rice-bran filtrate furnished 20 units of filtrate factor per gram.

RESULTS

Several complete series of riboflavin assays of the two sets of milk products were made. The results are shown in table 6 and summarized in table 7. Although fairly consistent values were obtained for the dried-milk samples, the variation in the results obtained for the whey samples was disappointingly large. At present the discrepancies in the whey assays cannot be explained. Other materials such as alfalfa meal have given a fair degree of reproducibility in repeated assays.

Recent unpublished studies indicate that the "unit of riboflavin" employed in this investigation corresponds to about 2.3 micrograms of synthetic d-riboflavin.

TABLE 5.—*Representative diets used (expressed in parts) and weight gains made in assay of dried-milk and whey products for riboflavin by the method of chick growth*

Constituent	Composition of diet No.—										
	96-86	96-87	96-88	96-89	96-90	96-91	96-92	96-100	96-101	96-102	96-103
Yellow corn meal	30	30	30	30	30	30	30	30	30	30	30
Cornstarch	20	17	22	22	22	22	22	23	23	23	23
Washed bran	10	10	10	10	10	10	10	10	10	10	10
Washed casein	19	19	20	21	21	21	21	22	22	22	22
Rice-bran filtrate, free from lactoflavin	7	7	7	7	7	7	7	7	7	7	7
Salt	1	1	1	1	1	1	1	1	1	1	1
Limestone	1	1	1	1	1	1	1	1	1	1	1
Bonemeal	1	1	1	1	1	1	1	1	1	1	1
Alfalfa hexane extract [1]	2	2	2	2	2	2	2	2	2	2	2
Cod-liver oil	3	3	3	3	3	3	3	3	3	3	3
Soybean oil											
Dried skim milk (November 1935)	6										
Dried whole milk (November 1935)		8.7									
Dried buttermilk (November 1935)			4								
Dried hydrochloric acid casein whey (November 1935)				3.9							
Dried rennet whey (November 1935)					3.9						
Dried sulphuric acid casein whey (November 1935)						4					
Dried lactic acid casein whey (November 1935)							3.9				
Whey adsorbate									1	2	2.5
Gain of chicks in 15-day assay period—grams	48	48	53	48	39	39	42	18	55	64	65
Gain over basal—do	30	30	35	30	21	21	25		37	46	47
Riboflavin content of supplement per gram—units	11	7	19	17	12	12	14		80	(2)	(2)

[1] Equivalent to 1 of alfalfa meal.
[2] Positive control diets.

TABLE 6.—*Riboflavin content of dried milk and whey samples prepared November 1935 and May 1936, as determined by biological assay with chicks*

Diet No.	Supplement (dried product)	Proportion of supplement in diet	When prepared	Period on diet	Gain on supplemented diet	Gain on basal diet	Gain on positive control diet	Riboflavin per gram of supplement
		Percent		*Days*	*Grams*	*Grams*	*Grams*	*Units*
93 2	Skim milk	5	November	20	49	22	71	11
93 3	Whole milk	7	do	20	51	22	71	8
93 4	Rennet whey	3.2	do	20	46	22	71	15
93 5	Hydrochloric acid casein whey	3.3	do	20	37	22	71	9
93 6	Lactic acid casein whey	3.2	do	20	35	22	71	8
93 7	Sulphuric acid casein whey	3.4	do	20	39	22	71	10
93 8	Buttermilk	5	do	20	62	22	71	16
96 16	Skim milk	5	do	14	28	9	53	9
96 17	do	5	May	14	34	9	53	11
96 19	Buttermilk	5	do	14	41	9	53	15
96 23	Skim milk	8	November	15	42	12	58	8
96 31	Lactic acid casein whey	5.1	May	15	33	12	58	9
96 32	Rennet whey	5.3	do	15	31	12	58	8
96 86	Skim milk	6	November	15	48	18	64	11
96 87	Whole milk	8.7	do	15	48	18	64	7
96 88	Buttermilk	4	do	15	53	18	64	19
96 89	Hydrochloric acid casein whey	3.9	do	15	48	18	64	17
96 90	Rennet whey	3.9	do	15	39	18	64	12
96 91	Sulphuric acid casein whey	4	do	15	39	18	64	12
96 92	Lactic acid casein whey	3.9	do	15	43	18	64	14
96 93	Skim milk	6	May	15	47	18	64	11
96 96	Hydrochloric acid casein whey	4.3	do	15	50	18	64	16
96 97	Rennet whey	4	do	15	35	18	64	9
96 98	Sulphuric acid casein whey	4.2	do	15	32	18	64	7
96 99	Lactic acid casein whey	3.8	do	15	36	18	64	10
96 116	Whole milk	8.3	do	14	62	18	75	9
96 117	Buttermilk	4	do	14	61	18	75	19
96 124	Sulphuric acid casein whey	4	November	14	47	9	70	16
96 125	Rennet whey	4	May	14	47	9	70	16
96 126	Skim milk	6	November	14	61	9	70	14
96 127	Hydrochloric acid casein whey	4	do	14	42	9	70	14
96 128	Rennet whey	4	do	14	57	9	70	19
96 129	Lactic acid casein whey	4	do	14	47	9	70	16
96 132	Skim milk	6	May	14	50	9	70	11
96 133	Hydrochloric acid casein whey	4	do	14	45	9	70	15
96 134	Sulphuric acid casein whey	4	do	14	48	9	70	16
96 135	Lactic acid casein whey	4	do	14	47	9	70	16

TABLE 7.—*Average values obtained for riboflavin content of dried-milk products in units per gram*

[Summarized results of table 6]

Dried-milk product	November 1935 samples					May 1936 samples		
	Units	*Units*	*Units*	*Units*	*Units*	*Units*	*Units*	*Units*
Skim milk	11	9	8	11	14	11	11	11
Whole milk	8	7				9		
Buttermilk	16	19				15	19	
Hydrochloric acid casein whey	9	17	14			16	15	
Rennet whey	15	12	19			8	9	16
Sulphuric acid casein whey	10	12	16			7	16	
Lactic acid casein whey	8	14	16			9	10	16

SUMMARY

A series of spray-dried milk products was prepared from a batch of milk in November 1935, and a similar series was prepared at the same place in May 1936. Each series consisted of whole milk, skim milk, buttermilk, rennet whey, hydrochloric acid casein whey, sulphuric acid casein whey, and lactic acid casein whey. Each series of samples was submitted to biological assay for the filtrate factor with chicks on a basal diet of heated natural foodstuffs. Any possible differences between the November and the corresponding May samples were not large enough to be detected by the method of assay. The descending order of potency for the November series was as follows: Rennet whey, sulphuric acid casein whey, lactic acid casein whey, hydrochloric acid casein whey and buttermilk, skim milk, whole milk. The order was the same for the May series except that buttermilk was slightly superior to hydrochloric acid casein whey. In rough figures, dried whey of various types furnished from 3 to 6 units of filtrate factor per gram; dried buttermilk, 3 to 4; dried skim milk, 2 to 3; and dried whole milk, 1 to 2.

Dried milk and dried whey are not concentrated sources of the filtrate factor, but it was possible to assay spray-dried milks and wheys with fairly good agreement between duplicate assays.

Milk products supply from two to five times as much riboflavin as the filtrate factor in terms of similarly computed chick units. Milk is valuable, therefore, in the chick ration as a source of riboflavin, since grains and grain byproducts present an opposite situation—that is, they tend to be very poor sources of riboflavin and to supply moderate amounts of the filtrate factor. Recent unpublished studies indicate that 1 chick unit corresponds to about 2.3 micrograms of synthetic d-riboflavin in terms of the methods employed in this investigation.

No difference within the limits of accuracy of the biological assay method used was found between November and May samples of milk products from the same locality.

Buttermilk was distinctly richer than skim milk in riboflavin.

Inconsistencies in the results made it impossible to say whether there were appreciable differences in the riboflavin content of whey samples prepared by different methods from the same batch of milk. As table 7 shows, fairly consistent results were obtained in repeated assays of skim milk, whole milk, and buttermilk; but the results with the various whey samples were irregular. At present the discrepancies in the whey assays cannot be explained. Other materials, such as alfalfa meal, have given a fair degree of reproducibility in repeated assays.

LITERATURE CITED

(1) HEIMAN, VICTOR, and CARVER, J. S.
1937. THE RELATIVE FLAVIN (VITAMIN G) CONTENT OF DRIED SKIMMILK, DRIED WHEY, AND DRIED BUTTERMILK. Poultry Sci. 16: 434–437.

(2) JUKES, THOMAS H.
1937. FURTHER OBSERVATIONS ON THE ASSAY, DISTRIBUTION, AND PROPERTIES OF THE FILTRATE FACTOR. Jour. Biol. Chem. 117: 11–20 illus.

(3) JUKES, THOMAS H.
1937. RECENT STUDIES OF VITAMINS REQUIRED BY CHICKS. Jour. Nutrition 13: 359–387.

(4) ———
1937. BIOLOGICAL ASSAY OF LACTOFLAVIN WITH CHICKS. Jour. Nutrition 14: 223–233.

(5) KEENAN, J. A., KLINE, O. L., ELVEHJEM, C. A., and HART, E. B.
1935. THE STABILITY OF VITAMINS B (B_1), G (B_2), AND B_4. Jour. Nutrition 9: 63–74, illus.

(6) LEPKOVSKY, SAMUEL, and JUKES, THOMAS H.
1936. THE EFFECT OF SOME REAGENTS ON THE "FILTRATE FACTOR" (A WATER-SOLUBLE VITAMIN BELONGING TO THE VITAMIN B COMPLEX AND PREVENTING A DIETARY DERMATITIS IN CHICKS). Jour. Biol. Chem. 114: 109–116.

RELATION OF SOIL TEMPERATURE TO CHLOROSIS OF GARDENIA[1]

By LINUS H. JONES

Assistant research professor of botany, Massachusetts Agricultural Experiment Station

INTRODUCTION

Normal leaves of gardenia are glossy and dark green in color. Immature leaves may be a lighter green, but they are glossy and quite green even as they open from the bud. During the winters of 1934–35 and 1935–36 a chlorotic condition of the leaves of gardenia growing under glass caused serious concern to Massachusetts growers. The malady in its early stages is characterized by a gradual decrease of green in the interveinal areas of the leaves. This decrease of green continues until a straw-yellow color has developed. The vein areas are the last to lose the green color. This type of chlorosis is first noted near the tips of shoots, i. e., newly developed and rapidly growing branches, and is followed by a similar chlorotic development in the leaves of the longer terminal branches. In later stages growth slows down and new leaves, as they appear, are relatively small and lack green color. Frequently, brown areas develop at the tip of the leaf and also in the margin.

Chlorosis is the visible effect of some cause, but it is by no means a criterion to establish a definite cause. Among the causes suggested for the chlorosis in question were the following: Deficiency in iron; soil reaction; unbalanced fertilizer ration; high concentration of calcium and sodium ions; nitrogen deficiency; lack of light; and mosaic diseases. A careful study of the environment and treatment of plants, some healthy and some chlorotic, indicated that none of these factors was involved. However, there did develop some evidence that a temperature factor was involved. Further investigation revealed that this temperature factor was localized in the soil.

METHODS

A preliminary test of the theory that soil temperature was the controllable factor in the chlorosis of gardenia was made by placing chlorotic potted gardenia [2] plants on a shelf over steam pipes. Check plants, not quite so chlorotic, were kept on a bench that did not have bottom heat. The test was started February 14 and was continued for 22 days. Records of air temperature about the two sets of plants showed the air temperature to be substantially the same. Soil-temperature records were taken daily at 9 a. m.

At about the twelfth day, the plants over the steam pipes showed a noticeable change in the tip leaves. The yellow color was disappearing as the healthy green color returned. These were young leaves and

[1] Received for publication February 11, 1938; issued November 1938. Published as Contribution No. 301 of the Massachusetts Agricultural Experiment Station.

[2] *Gardenia veitchii* was used in all the tests reported.

they developed well to their normal size. Leaves below the tip leaves were too old to expand to normal size, but they did regain their normal green color, which started from the base of the midrib and slowly expanded outwardly from the midrib. In general, the return of the green color was from the top of the plant downward and from the base of the leaf outward.

The check plants, less chlorotic at the start of the test, became exceedingly chlorotic. The newer leaves lacked green color as they unfolded and they developed to only a small fraction of their normal size. The record of the soil temperatures, shown in table 1, indicates the wide difference in this factor during the duration of the test.

TABLE 1.—*Soil temperature records of, and the temperature difference between, plants on benches with and without bottom heat*

Date	Bottom heat	Without bottom heat	Difference	Date	Bottom heat	Without bottom heat	Difference
	°C.	°C.	°C.		°C.	°C.	°C.
Feb. 15	23	18	5	Feb. 29	25	14	11
Feb. 16	26	17	9	Mar. 1	31	17	14
Feb. 17	28	18	10	Mar. 2	25	13	12
Feb. 18	23	15	8	Mar. 3	29	18	11
Feb. 19	22	11	11	Mar. 4	28	17	11
Feb. 20	22	10	12	Mar. 5	26	17	9
Feb. 21	23	11	12	Mar. 6	22	13	9
Feb. 22	31	17	14	Mar. 7	29	19	10
Feb. 23	23	14	9				
Feb. 24	24	14	10	Maximum	31	20	14
Feb. 25	30	18	12	Minimum	21	10	5
Feb. 26	30	20	10				
Feb. 27	27	17	10	Average	25.8	15.5	10.3
Feb. 28	21	13	8				

The recognition of soil temperature as a causal factor made it possible to check conditions in a greenhouse where chlorosis was well established. There were, however, plants or groups of plants that were healthy. Plants in containers near or over steam pipes had healthy dark-green leaves. Plants in benches, except at the ends of the house, were generally chlorotic. At one end of the house, steam entered the radiating system and at the other end, with extra piping and traps, steam was condensed and the water stored before being returned to the boiler. Soil-temperature readings in these healthy areas were markedly higher than those in the chlorotic areas. The lowest soil temperature in this particular house was in a bench about which steam seldom passed. It was in this bench that chlorosis always appeared first and became most severe.

The demonstration that this particular type of chlorosis could be obviated by raising the soil temperature, and the fact that chlorosis was prevalent at all low soil temperatures and absent at high soil temperatures, clearly indicated that somewhere within a wide range of soil temperatures there would exist a narrow band of temperatures below which chlorosis would be induced and above which it would not appear.

Experiments were, consequently, carried out in a constant soil-temperature apparatus [3] where it is possible to obtain and maintain a wide or narrow range of soil temperatures (fig. 1). The experiments

[3] FULLER, JAMES E., and JONES, LINUS H. THE INFLUENCE OF TEMPERATURE ON THE NITRATE CONTENT OF SOIL IN THE PRESENCE OF DECOMPOSING CELLULOSE. Soil Sci. 34: 337–350, illus. 1932.

were definitely planned not only to study the effect of soil temperature as the days grew shorter, but also to make a similar study as the daylight period lengthened. It was further planned that the influence



FIGURE 1.—Constant soil-temperature apparatus in which gardenias were exposed to a range of soil temperatures.

by the light factor in the apparatus must be overcome by inducing chlorosis by soil temperature at any and all available locations in the apparatus. The influence of previous exposure of the plant roots to

soil temperature was considered by establishing the plants at known soil temperatures before they were subjected to different soil temperatures.

EXPERIMENTAL RESULTS

EXPERIMENT 1, MARCH 25-JUNE 16

Hard plants from 3½-inch pots were shifted to galvanized-iron containers. There were two plants in each container and four containers at each temperature in the apparatus. The range of temperatures was from 8° to 32° C., the increment of increase being at 2° steps. The air temperature was maintained at about 21° by a thermostat control. However, for 14 days before the temperature adjustment was made, all plants were allowed to establish themselves at a soil temperature of 24°. The soil used was 4 parts good compost soil and 1 part peat.

Effect of Soil Temperature on the Plant

The first effect of soil temperature was noted at the two lower temperatures, 8° and 10° C. The plants wilted immediately as the temperature adjustment was attained. They recovered their turgidity at night, and maintained it in cloudy weather; but for 2 weeks they wilted in the sunshine, the tendency naturally decreasing as the period lengthened. Evidently root activity was depressed by the low temperature, and the roots could not absorb enough water to replace that lost by transpiration in periods of sunshine. This type of wilting, due to a lowered root temperature, has also been noted by Arndt,[4] who observed it in cotton plants grown in solution and in soil cultures.

The second effect was also noted at the two lowest temperatures where there occurred a rapid senescence of the oldest leaves, i. e., those nearest the soil surface. These leaves had become orange in color within 10 days and either dropped or slowly turned to the bronze brown of a dead leaf while remaining attached to the plant. This effect always developed in succeeding experiments and at the same soil temperature of 10° C. or less. At soil temperatures of 12° and above, there was no sign of rapid senescence, although now and then a leaf did turn orange, a perfectly normal occurrence. Undoubtedly the low soil temperature was the causal agent and not the shock of transplanting.

The third effect was the development of interveinal chlorosis. The transition from a healthy green to the early expression of yellow green in chlorosis was hardly noticeable, especially on bright days. It was best observed in cloudy weather. At the end of 40 days, it was apparent that interveinal chlorosis was present in all plants at soil temperatures of 18° C. and below. After 80 days there were a few leaves on plants at the soil temperature of 20° that showed interveinal chlorosis, but it was very slight. All chlorotic leaves at 18° and below became more chlorotic as the test progressed, and the degree of yellowing was directly associated with each drop in soil temperature.

Chemical Analysis of Plant Parts

The leaves and terminal twigs of healthy and chlorotic plants were harvested at the end of 83 days, dried for 48 hours at a temperature of

[4] Arndt, Charles H. Water absorption in the cotton plant as affected by soil and water temperatures. Plant Physiol. 12: 703–720. 1937.

73° C., and chemically analyzed, with the object of determining whether an essential element was lacking. The results of the analysis are shown in table 2.[5]

TABLE 2.—*Chemical analysis of leaves and twigs from healthy and chlorotic gardenia plants*

Constituent	Leaves		Terminal twigs	
	Chlorotic; 20° C. and lower	Healthy; 24° C. and higher	20° C. and lower	24° C. and higher
	Percent	*Percent*	*Percent*	*Percent*
Ash	8.82	6.93	8.69	8.86
Organic matter	91.18	93.07	91.31	91.14
	100.00	100.00	100.00	100.00
Phosphoric acid (P_2O_5)	.46	.46	.60	.99
Nitrogen	3.36	2.84	2.81	2.33
Chlorine (as chlorides)	.16	.16	.10	.10
Sulphuric acid (SO_3) as sulphates	.55	.27	.27	.27
Potassium oxide (K_2O)	2.59	1.50	1.47	1.73
Sodium oxide (Na_2O)	.98	.43	.64	.44
Iron and aluminum oxides (Fe_2O_3. Al_2O_3)	.09	.11	.09	.09
Calcium oxide (CaO)	1.14	1.06	2.87	2.37
Magnesium oxide (MgO)	.54	.60	.78	.75
Manganese (Mn)	.008	.006	.005	.0024
Insoluble matter	.29	.47	.13	.19

There do not appear in the tabulation of the chemical analysis sufficient differences to warrant the interpretation that an essential element is lacking in the chlorotic plants. As a matter of fact, some of the elements are more concentrated in the chlorotic plants, a condition commonly found in diseased or unhealthy plants. While it is possible that at the lower soil temperatures, absorption from the soil by the plant or translocation within the plant may have been depressed, the fact remains that the plants were returned to a normal development by simply raising the soil temperature, which indicated the presence, in adequate quantity, of the necessary chemical constituents.

EFFECT OF SOIL TEMPERATURE ON SIZE OF LEAF

In due time it was evident that the effect of soil temperature expressed itself not only in the degree of chlorosis, but also in the degree of growth and size of leaf. It is of particular interest to note that the size of the leaf was not at all influenced by the air temperature, which was maintained fairly constant the entire length of the apparatus while the soil temperature was the variable. This variable of soil temperature was related to leaf size as measured by length of leaves.

With the limited number of plants it was possible to choose for measurement 10 leaves at each temperature. These leaves were all developed and matured after the experiment was started. The measurements were made 50 days after the temperatures were altered. The averages of the measurements at each soil temperature were taken and graphed as shown in figure 2. These measurements show that the length of the leaf was increased by a rise in soil temperature. No measurable growth took place at the lowest temperature, 8° C., although the plant attempted to form new leaves. These soon withered

[5] This analysis was made by Prof. H. D. Haskins of the Massachusetts Agricultural Experiment Station.

and died in the early effort of expanding. Figure 2 shows a close correlation between size of leaf and chlorosis, indicating that the root medium must supply not only mineral nutrients and water for the plant, but also a temperature environment at which the above-ground portions may carry on their normal functions. In figure 2 the leaves at soil temperature 22° are blocked off from both chlorotic and healthy areas because, though their length was that of healthy leaves, there were slight traces of chlorosis.

EXPERIMENT 2, SEPTEMBER 22–MAY 24

Experiment 2 was planned in two parts: Part 1, to repeat experiment 1 as the days were growing shorter (the days were growing longer in experiment 1); part 2, to shift the temperatures at a suitable time to determine whether the position of the plants in the apparatus

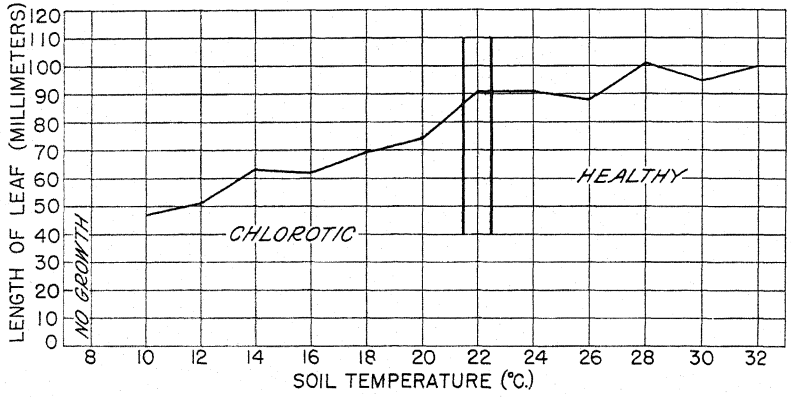


FIGURE 2.—Effect of soil temperature on length of leaves.

was in any way connected with the physiological disturbances as noted in the first experiment.

The same soil temperatures were used except the one at 8° C., which in experiment 1 proved too low for growth. The plants used in this experiment were in a soft condition, vigorous and rapidly growing, in 5-inch clay pots. They were properly acclimated in the apparatus and the range of soil temperatures adjusted.

The development of chlorosis was first evident, after 27 days, in the plants at the lowest soil temperature, 10° C. At the end of 60 days there was the same general distribution of chlorosis as in experiment 1. All new leaves at soil temperatures of 10° and 12° were pale yellow. At 14°, 16°, and 18° the chlorosis was characterized by interveinal yellowing, which was less at each rise in soil temperature. All plants at 22° and above were a normal healthy green, but at 20°, the same uncertain point as in experiment 1, the color of the foliage was normal except on newly formed basal shoots. Such shoots emerge either from below the soil line or just above it. It is possible that the shoots themselves had not developed sufficient tissue to insulate them from the effects of a cool temperature emanating from the soil. Chroboczek [6] has demonstrated that lowered temperatures

[6] CHROBOCZEK, EMIL. A STUDY OF SOME ECOLOGICAL FACTORS INFLUENCING SEED-STALK DEVELOPMENT IN BEETS (BETA VULGARIS L.). N. Y. (Cornell) Agr. Expt. Sta. Mem. 154, 84 pp., illus. 1934.

about the base of a growing point alter the physiological function of the resulting growth.

Twelve weeks (September 22–December 15) after experiment 2 was started and when no further chlorotic effects could be expected, the temperatures, not the plants, were shifted to make sure that the position of the plants in the apparatus was not the determining factor of chlorosis. The plan of the shift and the condition of the plants at the time of shift and at the end of the experiment are shown in figure 3.

The only immediate effect observed from this sudden shift in temperature of the soil was the wilting, at soil temperatures of 10° and 14° C., of the plants which a few hours previously were at soil temperatures of 32° and 28°, respectively. Wilting occurred for a few days, but the plants eventually recovered their turgidity.

Distribution of Temperatures in Apparatus														
	(West)							(North)	(East)					24 weeks
	10° C.	12° C.	14° C.	16° C.	18° C.	20° C.		22 °C.	24° C.	26° C.	28° C.	30° C.	32° C.	
Condition at end of 12 weeks	Chlorotic								Green					
Plants unchanged; temperatures shifted.														
	32° C.	12° C.	28° C.	16° C.	24° C.	22° C.	20° C.	18° C.	26° C.	14° C.	30° C.	10° C.		
Condition at end of second 12 weeks	Green	Chlorotic (very)	Green	Chlorotic (very)	Green	Green	Light green	Chlorotic (medium)	Green	Chlorotic (medium)	Green	Dark green		

Figure 3.—Diagram to illustrate the effect of soil temperature on chlorosis of gardenia plants regardless of their position in the soil-temperature apparatus.

The plants which had been subjected to low temperatures for 12 weeks (soil temperatures of 10° and 14° C.) were very chlorotic. After they were subjected to higher temperatures (soil temperatures of 32° and 28°), they began to show signs of a return to green color at the thirteenth day. At the end of 56 days, plants which had been green at fairly high soil temperatures (28° and 24°) showed interveinal chlorosis at the changed temperatures of 14° and 18°. The new leaves of these plants were developing with a yellow color instead of the normal green.

Chlorosis did not develop at the lowest soil temperature, 10° C. This exception by no means distorts the previous findings that a low soil temperature will induce chlorosis. This particular group of plants had been growing for 12 weeks at the very high soil temperature of 32°. Under these conditions of excellent root and above-ground environments, the plants were enabled to take advantage of all synthetic processes more intensely than any other group of plants in the experiment. When the temperature of the soil was dropped to 10°, all vegetative growth ceased and the leaves (there were no new leaves

produced after the drop in temperature), instead of becoming chlorotic, became a dark green. The immense supply of food material built up in the 12-week period of high soil temperature was not used in new growth after the temperature was dropped, but evidently was influential in altering the vegetative habit of development to a reproductive habit, for the plants produced a tremendous set of buds, practically every terminal bud developing into a flower bud. There was an average of 34 flower buds per plant, and most of them developed into flowers. The low temperature did not create bud drop. The same alteration from a rapid vegetative growth to reproductive development occurred in the plants which had been growing 12 weeks at 28° and were dropped to 14°, although slight vegetative growth did take place and chlorosis appeared. To a much less extent, the development of flower buds was apparent in the plants originally at the soil temperature of 24° and dropped to 18°. There exists a definite consistency in the relation of high soil temperatures being dropped to fairly low soil temperatures and bud formation. Thus the plants grown at 32°, 28°, and 24° and dropped to 10°, 14°, and 18° respectively (soil temperatures of 30° and 26° were not changed) all produced buds, the number of which was seemingly tied up with the severity of shock to the plant as measured in degrees of temperature dropped. (As this study is confined to chlorosis, the subjects of bud set and bud drop, both little understood, will not be discussed further in this paper.)

In general, the results in experiment 2 were similar to those in experiment 1. They show that soil temperature is the major influence in inducing chlorosis or in causing its disappearance, regardless of time of year. By shifting the position of the temperatures in the apparatus, it was shown definitely that no experimental error due to a light or air environment factor had escaped attention.

It is important to note that those plants that had been at the highest soil temperatures in the first part of experiment 2 developed chlorotic leaves but slowly when the temperature was dropped to a medium level in the second half of the experiment. The results from the second half of experiment 2, while following the trends of the first part, and also the trends of experiment 1, show a delay in developing a chlorotic response, which clearly is related to the soil temperatures and length of time exposures to these soil temperatures before the temperature was dropped. This delay in the onset of chlorosis is also noted in greenhouses, where it has been observed that newly set plants become chlorotic much earlier than plants that have been benched for a year.

EXPERIMENT 3, JUNE 16-NOVEMBER 1

Since, in experiment 2, it was shown that the rate of onset of chlorosis was related to previous soil temperatures and length of exposure to them, it was planned that all sets of plants should be grown over a considerable period at the high soil temperature of 32° C. before being subjected to the range of soil temperatures. Young plants from 2½-inch pots, two in each container, were grown for 90 days at this high soil temperature. Soil temperatures were then adjusted with the increment of increase at 3° instead of 2° in order to make more efficient use of the refrigerating system. However, this increase in the increment did not interfere seriously in making

comparisons of results in experiment 3 with results in experiments 1 and 2.

At the end of 30 days, chlorosis was most intense at the soil temperature of 13° and less intense at 16° C. No chlorosis was apparent at soil temperatures of 19° and above. At the end of 45 days, chlorosis had increased at soil temperatures of 13° and 16°, with a slight indication at 19° and a trace at 22°.

Figure 1 shows the plants in the constant soil-temperature apparatus, and figure 4 shows the chlorotic plant (13°), the healthy plant (32°), and the very dark-green plant (10°). As in experiment 2, the plants at the soil temperature of 10° C. developed no symptoms of chlorosis, but turned a darker green.

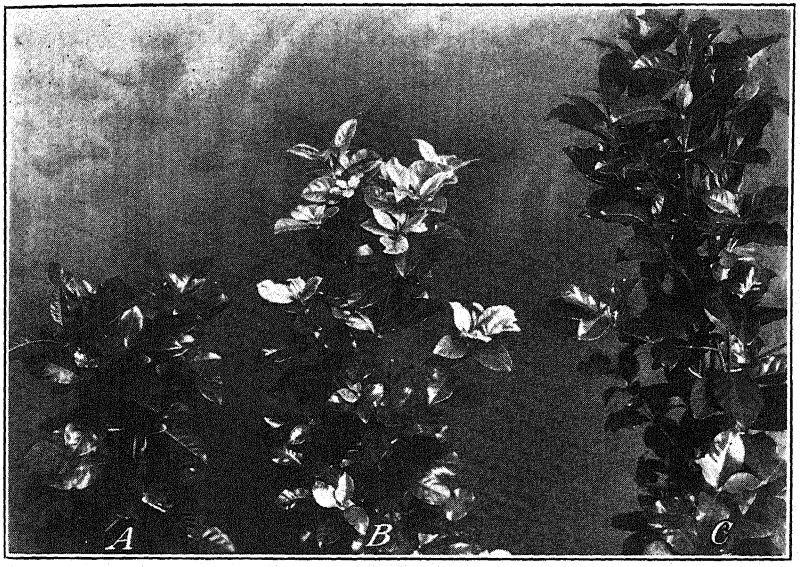


FIGURE 4.—Chlorotic gardenia plant, 13° C. (*B*); healthy plant, 32° (*C*); dark-green, nongrowing plant, 10° (*A*).

In experiment 3 a definite attempt was made to corelate the changes in leaf color with growth-vegetative activity. At the time the temperatures were adjusted to the desired range, india-ink markings were placed upon plant stems at all soil temperatures. Enough of these markings were made so that 10 could be chosen as representing typical growth at each temperature. The measurements of increase in length of stem were made at the 45-day period. The averages of these measurements are recorded in table 3, along with the color response as affected by soil temperature. It will be observed that there was no measurable growth at the lowest soil temperature, 10° C. Otherwise, there seems to exist a relation between increase of elongation of stems and the lessening of chlorosis. Although a correlation may exist, and both are traceable to soil temperature as the causal agent, it is difficult to prove that chlorosis is due to a retardation of growth or vice versa. There was no growth at the soil temperature of 10°, neither was there any chlorosis.

TABLE 3.—*Effect of soil temperature on distribution of chlorosis and rate of growth from September 13 to October 27*

Soil temperature (° C.)	Growth	Extent of chlorosis	Soil temperature (° C.)	Growth	Extent of chlorosis
	Centimeters			*Centimeters*	
10	0.0	Dark green.	22	11.6	Trace of chlorosis.
13	4.8	Very chlorotic.	25	12.3	Green.
16	7.7	Chlorotic.	32	13.9	Do.
19	10.5	Slightly chlorotic.			

DISCUSSION

The fact that soil temperature not only induces and controls chlorosis of gardenia, but also affects the above-ground parts in size of leaf, rate of growth, and alteration of the vegetative and reproductive phases, and even produces wilting when lowered to 10° C. or less, emphasizes an important factor for consideration in the science of ecology. While it is customary to consider the effects of temperature on the basis of air-temperature readings, and while there is undoubtedly a correlation of plant response with air temperature, nevertheless, as these experiments show, the correlation with soil temperature may be even closer.

In field and forest no particular control can be exercised on adjustments of soil temperature. However, it might prove profitable to study the various responses that are traceable to soil temperatures.

Under greenhouse conditions, where some degree of control can be exercised, it becomes apparent that the practice of controlling air temperature may have some virtue, but it seems possible that plant response can be guided better by an exact consideration of soil temperature. While sunshine and clouds may alter the air temperature quickly, neither has an immediate effect on soil temperature, which lags several hours in attaining its maximum and minimum in relation to the same extremes of air temperature. This lag is not consistent or easily predictable, since the amount of moisture in the soil is not a constant and may vary considerably from immediately after watering until the watering is repeated. The amount of water applied and its temperature are also factors which may lower the soil temperature only slightly in summer and early autumn, but will lower it considerably in midwinter and early spring. Heating the water to be applied is sometimes practiced, but no recommendations have heretofore been made that soil-temperature readings be made in connection with watering or other greenhouse practices. Depth of soil is also important and one would expect a slower alteration of soil temperature in ground beds than in raised benches.

While the tests discussed in this paper have dealt with *Gardenia veitchii*, it has been observed that the varieties with larger leaves and larger flowers are considerably more susceptible to the influence of soil temperature on chlorosis. It has been observed also that plants benched in August develop chlorosis more quickly than those benched the previous August. Age and length of exposure to high summer temperatures are factors which condition the gardenia plant to resist the early soil-temperature influence on inducing chlorosis.

SUMMARY AND CONCLUSIONS

The discovery that a high soil temperature would dissipate the chlorosis of *Gardenia veitchii* suggested the testing of the effects of soil temperature on this plant. The work was conducted in a constant soil-temperature apparatus capable of supplying a range of soil temperatures at any time of the year. The work was repeated sufficiently to warrant the following statements.

Chlorosis was induced at a soil temperature of 18° C. and less, and developed more intensely directly as the soil temperature was lower.

At soil temperatures of 20° and 22° C. there were traces of chlorosis, but it did not increase in intensity as time went on.

A chemical analysis of leaves and terminal twigs from healthy and chlorotic plants did not indicate a deficiency in nutrient elements. It did show, in the case of leaves, analytical results characteristic of unhealthy plants.

Change in the length of the day did not affect the onset of chlorosis.

The time required to induce chlorosis varied with the condition of the plants when the temperatures were lowered. Hard plants growing at medium temperatures developed chlorosis more slowly than soft plants grown at high temperatures.

The position of the plants in the constant soil-temperature apparatus (daylight and possibly air-temperature factors) had no influence on the induction or dissipation of chlorosis.

A sharp rise in soil temperature maintained for 13 days was sufficient to initiate the gradual return of a healthy green color.

There was an inverse correlation between the rate of growth and the intensity of the development of chlorosis.

Soil temperature is a factor affecting the size of the leaf, the length of the leaf increasing as the temperature is raised.

At the lower soil temperatures, 8° and 10° C., the plants become chlorotic if they have been growing slowly. If they have been growing rapidly at a high temperature, they do not become chlorotic when the temperature is lowered, but develop a very dark green color and cease to grow at all.

Plants growing at a fairly high soil temperature wilt when the temperature is dropped suddenly to 8° and 10° C., but eventually recover their turgidity.

An effect of lowering the soil temperature to 10° C. or less is the rapid senescence of the oldest leaves.

Plants that have been at a high soil temperature and are subjected to a low temperature grow little, but set buds. On the other hand, plants that have been at a low temperature and are subjected to a high temperature grow vigorously, but set no buds. It is evident that soil temperature is a factor that may alter the physiological phases of vegetative and reproductive development.

102953—38——5

RESISTANCE IN THE RED RASPBERRY TO THE MOSAIC VECTOR AMPHOROPHORA RUBI KALT.[1]

By GLENN A. HUBER, *plant pathologist, Western Washington Experiment Station* and C. D. SCHWARTZE, *assistant horticulturist, Washington Agricultural Experiment Station and horticulturist Western Washington Experiment Station*[2]

INTRODUCTION

The presence of red raspberry mosaic[3] in Washington has been discussed by Jones and Baur (*3*).[4] This disease is gradually spreading to new localities, and is of major importance in western Washington because of the high susceptibility of the principal commercial variety, Cuthbert, to infection.

Rankin (*5*), in New York, observed that certain red raspberry varieties, such as Herbert and Latham, escaped infection to a greater extent than others. Slate and Rankin (*7*) state that the reason for the slower spread in one variety than in another is not known but "reflects some varietal character in relation to aphid feeding that causes it to escape infection even when aphids arrive on it from diseased plants." Winter (*8*), in Minnesota, found that the insect vectors of mosaic showed a decided preference for certain varieties. He reported that the Herbert variety exhibited marked resistance to *Amphorophora rubi* Kalt., whereas Latham and King were susceptible. Field counts made in Michigan by Bennett (*1*) showed high aphid populations on Latham, King, St. Regis, and the wild red raspberry, but smaller populations on Cuthbert. Cooley (*2*), in New York, observed a widely fluctuating population of *A. rubi* on cultivated raspberries, in contrast to the steady population on most of the wild red raspberry stocks. He states: "This is no doubt a result of the fact that the wild red raspberries are usually growing in protected and shaded locations where the effects of high winds, driving rains, and summer heat are somewhat abated."

It is generally accepted that certain species of red raspberry aphids are the infective agents in mosaic transmission. In western Washington, *Amphorophora rubi*,[5] (*4*) is the most prevalent and widely distributed of the raspberry aphids and has been shown repeatedly to transmit mosaic. Its active nature and abundance throughout the summer months in contrast to other species that are found occasionally indicate that it is the principal vector of mosaic in red raspberry varieties in this section.

While studying mosaic symptoms and the spread of the disease on various red raspberry varieties in experimental plots and commercial

[1] Received for publication March 19, 1938; issued November 1938. Published as Scientific Paper No. 387, College of Agriculture and Experiment Station, State College of Washington. A brief paper including a part of the data herein reported has been published (*6*).

[2] The writers express their appreciation to A. J. Hanson, entomologist, Western Washington Experiment Station, for suggestions received during the progress of the investigation.

[3] Reference is made in this paper only to the virus disease known as "green" or "severe" red raspberry mosaic.

[4] Italic numbers in parentheses refer to Literature Cited, p. 632.

[5] The writers are indebted to Dr. G. F. Knowlton, associate entomologist, Utah Agricultural Experiment Station, for the identification of this species.

plantings, the writers observed that *Amphorophora rubi* was more abundant on some varieties than on others and was absent from the variety Lloyd George. This indication of resistance to *A. rubi* in the Lloyd George variety appeared to have special significance because of the superiority of this variety in breeding for fruit quality. Examination of young hybrids resulting from crossing Cuthbert, Lloyd George, and other named varieties revealed that some harbored large numbers of aphids, others small numbers, and a few none at all, suggesting that resistance to *A. rubi* may be transmitted in hybridization.

Following these preliminary observations, a more thorough investigation of aphid resistance in named varieties and hybrids was undertaken by means of both greenhouse experiments and field observations. The results obtained from 1935 to 1937, inclusive, are presented in this paper.

MATERIALS AND METHODS

The named varieties of red raspberry (*Rubus strigosus* Michx.) used in greenhouse experiments were from the Western Washington Experiment Station experimental plantings, namely, the variety trial plots maintained for breeding purposes, an isolated plot for foreign introductions, and an isolated planting maintained for raspberry disease investigations. Field studies were made in the same plots and additional data were obtained from commercial plantings throughout the Puyallup Valley. All of the plots from which data were obtained had been established for a sufficient length of time to insure aphid infestation.

The investigation of aphid resistance in hybrid raspberries utilized materials previously developed in breeding for winter resistance. For greenhouse studies, young vigorous "sucker" plants, obtained from each hybrid in the field, were transferred to 5-inch pots. In field studies with hybrids, observations were made in duplicate "increase" plots in which each hybrid selection was represented by 6 to 50 plants. These plantings had been established 2 or more years before the aphid counts were made.

In the greenhouse, usually five plants of each variety or hybrid were tested. All plants were tested for aphid resistance either by placing a single potted plant in a voile cloth cage and infesting the cane tips with aphids, or by confining the aphids to the tips of succulent canes in ventilated cellophane bags (fig. 1). The aphids, *Amphorophora rubi*, used in the 1935 experiments were collected from station plots. Those used in 1936 and 1937 experiments were reared in the greenhouse on Cuthbert plants confined in voile cloth cages (fig. 2). The original colony was established from a single apterous stem mother. Transfers were made from one plant to another with small camel's-hair brushes.

In the field, in order to obtain a comparative count of the aphid populations on varieties and hybrids, 10 canes of each, selected at random throughout the plantings, were examined. Because of varying lengths of canes and because the aphids were found more commonly on the upper leaves and tips of the canes, the upper 10 leaves, in addition to the unfolding leaves and cane tips, were examined and the aphids counted.

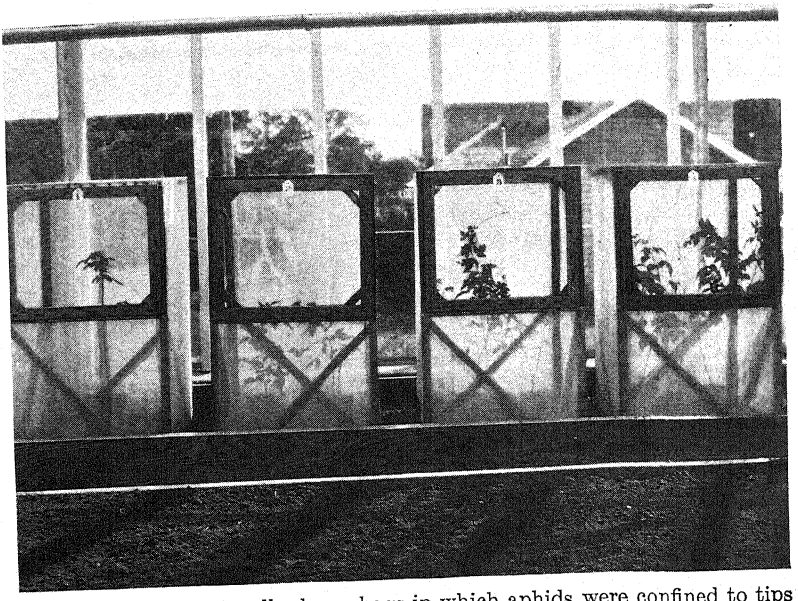


FIGURE 1.—Ventilated cellophane bags in which aphids were confined to tips of canes of red raspberry varieties and seedlings.

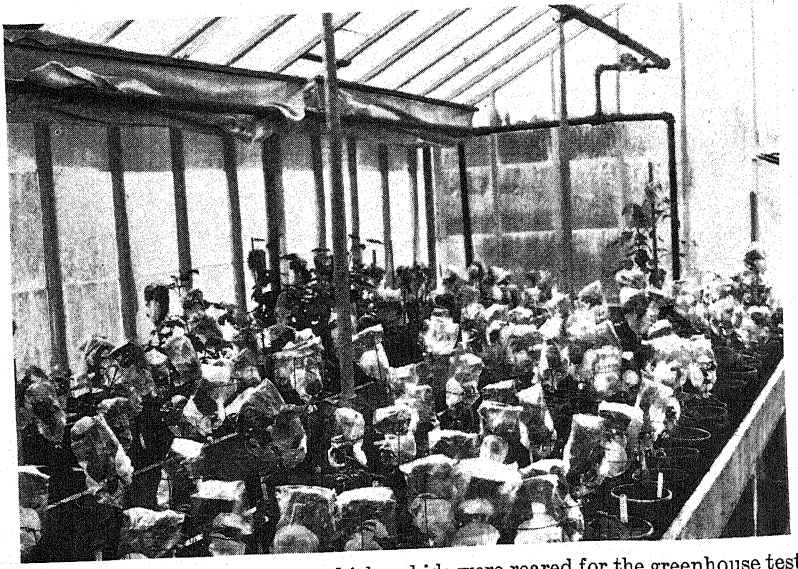


FIGURE 2.—Voile cloth cages in which aphids were reared for the greenhouse tests.

RESISTANCE IN VARIETIES

GREENHOUSE EXPERIMENTS

Preliminary observations on red raspberry varieties in various plantings showed such marked differences in the number of aphids present that two experiments were conducted in the greenhouse in 1935 to determine the behavior of *Amphorophora rubi* on several varieties under controlled conditions.

In the first experiments, young succulent canes of 11 varieties were cut in the field, immediately placed in water, and taken to the greenhouse. Here they were transferred to Erlenmeyer flasks filled with water and so arranged that each flask contained one cane of each of two varieties. All possible varietal combinations were made. The canes in each flask were fastened together at the base and the leaves were allowed to intermingle. All aphids were removed from the canes and 10 aphids, second and third instars, were placed on the tip leaves of each cane. The aphids were collected in the field from varieties different from those on which they were placed. The water in the flasks was changed twice daily, and the temperature in the greenhouse was maintained at 70° to 75° F.

After 7 days the aphids on each cane were counted. Each leaf was carefully removed when the count was made so as to distrub the aphids as little as possible, thus minimizing movement from one variety to another during the process of counting. The results of the experiment are given in table 1.

On the varieties Chief, Cuthbert, King, Latham, Marlboro, Newman, and Viking the aphid population increased abundantly, while on Antwerp, Herbert, Lloyd George, and Newburgh it increased only slightly or not at all. Paired varieties of the former group show in general a marked increase of *Amphorophora rubi* on both, while in paired varieties of the latter group there was a marked decline in numbers of aphids with one exception, the Newburgh-Herbert combination. When varieties of each group were paired the number of aphids increased on varieties of the former group and decreased on those of the latter, with few exceptions. The exceptionally high counts on Marlboro in the Lloyd George-Marlboro pair and on Newman in the Lloyd George-Newman pair suggests that the aphids moved from the Lloyd George to Marlboro and Newman.

TABLE 1.—*Population of Amphorophora rubi on paired canes of red raspberry varieties* [1] *held in Erlenmeyer flasks in the greenhouse for 7 days*

Variety	Antwerp		Chief		Cuthbert		Herbert		King		Latham		Lloyd George		Marlboro		Newburgh		Newman	
Chief	87	1	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----
Cuthbert	89	2	58	38	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----
Herbert	7	4	15	34	1	[2] 20	----	----	----	----	----	----	----	----	----	----	----	----	----	----
King	47	5	39	20	73	43	51	9	----	----	----	----	----	----	----	----	----	----	----	----
Latham	69	0	61	61	24	[2] 27	11	7	15	89	----	----	----	----	----	----	----	----	----	----
Lloyd George	0	3	3	46	1	57	0	5	2	54	3	31	----	----	----	----	----	----	----	----
Marlboro	86	7	68	86	55	21	75	12	29	29	39	5	101	0	----	----	----	----	----	----
Newburgh	13	0	23	41	2	57	15	20	28	78	13	25	1	1	9	42	----	----	----	----
Newman	97	4	36	99	27	42	28	7	76	83	21	74	123	0	27	140	21	6	----	----
Viking	47	0	54	41	19	31	28	7	24	22	107	15	61	0	96	33	63	13	61	14

[1] The figures in the column at the left under each variety give the number of aphids on the variety listed in the stub; the figures in column at the right give the number of aphids on the variety at the top of the column.

[2] Larvae of syrphid fly present on the Cuthbert cane only.

The few aphids that remained on the Lloyd George canes were adults, indicating that reproduction had not occurred. The populations on Antwerp were small, but the presence of first instars indicated that reproduction had taken place. The number of instars found on Herbert and Newburgh showed that reproduction had taken place to a slightly greater extent than on Antwerp.

The behavior of *Amphorophora rubi* when confined to Antwerp, Herbert, Lloyd George, and Newburgh indicated that these varieties possess a marked degree of resistance to this mosaic vector.

In the second experiment, young succulent potted plants of the same varieties used in the first experiment were placed in the open insectary under voile cloth cages, each containing a single aphid-free plant. Twelve adult aphids were placed on each plant. The aphid counts on the different varieties after 7 days were as follows: Antwerp, 5; Chief, 77; Cuthbert, 74; Herbert, 42; King, 86; Latham, 88; Lloyd George, 0; Marlboro, 69; Newburgh, 23; Newman, 109; and Viking, 73. These data also indicated a marked resistance in Antwerp and Lloyd George, and a less marked resistance in Herbert and Newburgh, in contrast to the susceptibility of the other varieties.

During the growing season of 1936, an experiment was conducted in which a young vigorous plant of each of 15 varieties was placed in a cage by itself, and 10 aphids, second and third instars, were distributed on the tip leaves of each plant. Counts of the aphid populations were made 16 days later. The results showed more than 200 aphids (adults and instars) on each of Cayuga, Chief, Cuthbert, June, King, Latham, Marlboro, Newman, Preussen, Seneca, and Viking. No aphids were found on Herbert, Lloyd George, or Newburgh, and the population on Antwerp was small. The experiment was repeated by placing 25 aphids, second and third instars, on a caged plant of each of these four varieties. Counts of aphids made 14 days later were as follows: Antwerp, 32; Herbert, 6; Lloyd George, 0; and Newburgh, 21.

Since no aphids were found on the Lloyd George in the final count in each of the preceding experiments, a third test was conducted on this variety. Aphids were confined by means of ventilated cellophane bags to the tips of succulent canes on six potted Lloyd George plants. A leaf harboring numerous aphids of all stages was placed on the tip of the cane in each bag. When examined 16 days later, the aphids in all bags were dead.

During the growing season of 1937, the 15 varieties tested in 1936 were retested. The results confirmed those obtained in 1936. The experiment also included 10 additional varieties. *Amphorophora rubi* failed to maintain its population on three of these, namely, Indian Summer, Pyne Imperial, and Pyne Royal. These three varieties were tested a second time, and again the aphids failed to maintain their populations. Aphids fed and reproduction took place on the other seven varieties: Katherine, Laxton Bountiful, Laxton Renown, Marcy, Red Cross, Rote Riesen, and Taylor. Marcy showed some resistance in that the aphid population was low in comparison with that on the other varieties.

Table 2 summarizes the results of experiments conducted in the greenhouse during 1936 and 1937 for aphid resistance in red raspberry varieties based on their relative susceptibility.

TABLE 2.—*The reaction of 25 varieties of red raspberry to Amphorophora rubi under greenhouse conditions during 1936 and 1937*

Variety	Susceptible [1]	Partly resistant [2]	Resistant [3]	Variety	Susceptible [1]	Partly resistant [2]	Resistant [3]
Antwerp		(*)		Marcy		(*)	
Cayuga	(*)			Marlboro	(*)		
Chief	(*)			Newburgh		(*)	
Cuthbert	(*)			Newman	(*)		
Herbert		(*)		Preussen	(*)		
Indian Summer			(*)	Pyne Imperial			(*)
June	(*)			Pyne Royal			(*)
Katherine	(*)			Red Cross	(*)		
King	(*)			Rote Riesen	(*)		
Latham	(*)			Seneca	(*)		
Laxton Bountiful	(*)			Taylor	(*)		
Laxton Renown	(*)			Viking	(*)		
Lloyd George			(*)				

[1] The asterisk (*) indicates that the number of aphids was greater than 20 times the original number placed on the plant.
[2] The asterisk (*) indicates that the number of aphids was less than 5 times the original number placed on the plant.
[3] Aphids failed to reproduce and maintain a population.

FIELD OBSERVATIONS

Aphid counts were made in the field plots, July 2, 1935, on the 11 red raspberry varieties used in the first greenhouse experiment. The average number of aphids per cane was as follows: Antwerp, 11.5; Chief, 129.9; Cuthbert, 57.6; Herbert, 11; King, 66.5; Latham, 90.7; Lloyd George, 0; Marlboro, 69.6; Newburgh, 13.1, Newman, 86.3; and Viking, 87.

Aphid counts were made on the same varieties on several dates in 1936, as shown in table 3.

TABLE 3.—*Range* [1] *and average number of Amphorophora rubi on varieties of red raspberry in experimental plots, 1936*

Variety	May 25		June 9		June 25		July 7		July 25		Aug. 12		Aug. 28	
	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average
Antwerp	0–2	0.8	1–8	0.8	0–2	0.2	0–1	0.1	0–5	1.8	0–26	4.9	0–8	2.0
Chief	2–42	19.4	9–43	21.7	8–104	42.7	13–82	39.4	[2]3–108	41.1	16–64	44.9	6–55	22.4
Cuthbert	0–2	.5	0–7	2.6	0–16	5.0	0–11	5.0	[2]0–30	10.8	1–61	16.0	0–29	10.0
Herbert	-----	-----	-----	-----	0–3	.3	0–10	1.4	[2]0–6	1.8	0–6	2.4	0–6	.9
King	-----	-----	-----	-----	9–46	23.3	0–32	9.4	0–29	10.9	3–36	19.5	0–7	2.7
Latham	0–28	13.4	1–33	9.1	2–27	14.7	6–28	16.4	[2]4–49	23.1	3–54	21.9	0–26	6.2
Lloyd George	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Marlboro	0–8	3.7	0–18	5.1	1–9	4.6	4–21	7.7	4–42	16.8	1–13	6.1	0–4	1.7
Newburgh	-----	-----	-----	-----	0–6	2.0	0–13	4.7	[2]0–9	4.6	1–8	5.1	0–6	1.8
Newman	3–13	6.1	3–60	16.6	4–17	10.1	2–48	14.7	1–26	12.5	3–26	10.9	0–21	5.9
Viking	1–29	8.9	0–20	6.8	0–39	16.0	2–24	12.7	3–83	32.8	10–89	33.7	1–63	15.8

[1] The figures under range indicate the lowest and the highest aphid counts on individual canes.
[2] Count made July 23.

No aphids were found feeding on the Lloyd George at any time during the season.[6] Populations were generally low on Antwerp, Herbert, and Newburgh. The highest population was found on Chief. In general, the aphid population was greatest during the latter part of July and the fore part of August. According to the counts, aphids were not as numerous at any time during 1936 as in 1935.

[6] An occasional winged individual was found on the Lloyd George, which probably landed there accidentally while in flight.

Aphid counts [7] were made in commercial plantings of 36 different growers in the Puyallup Valley during July and early in August 1936. On the Cuthbert variety there was a great variation in aphid populations in different plantings even though the counts were made on the same date. For instance, counts made July 30 in one field showed an average of 36.7 aphids per cane, while in another field, located within a distance of one-half mile, there was an average of 4.1 aphids per cane. On Marlboro the aphid populations were small in the 12 plantings examined. An average of 4.4 aphids per cane was the heaviest infestation observed. Two Antwerp plantings were examined. One showed an average of two aphids per cane on July 8 and the other, examined July 28, showed none. On Lloyd George no aphids were observed in the six plantings examined.

During the growing season of 1937, a systematic aphid count was made in the experimental plots on 25 varieties. They were examined at intervals of 2 weeks or oftener, beginning May 14 and continuing until August 17. The results of the counts are summarized in table 4.

TABLE 4.—*Relative resistance of red raspberry varieties to Amphorophora rubi in experimental plots during 1937*

Variety	Times examined	Susceptible [1]	Partly resistant [2]	Resistant [3]
Antwerp	23		(*)	
Cayuga	12	(*)		
Cuthbert	21	(*)		
Herbert	23		(*)	
Indian Summer	10			(*)
June	5	(*)		
Katherine	5	(*)		
King	10	(*)		
Latham	17	(*)		
Laxton Bountiful	5	(*)		
Laxton Renown	5		(*)	
Laxton Reward	5	(*)		
Lloyd George	26			(*)
Marcy	11		(*)	
Marlboro	13	(*)		
Newburgh	29	(*)		
Newman	7	(*)		
Preussen	5	(*)		
Pyne Imperial	5			(*)
Pyne Royal	5			(*)
(St. Regis) Ranere	12	(*)		
Red Cross	5		(*)	
Rote Riesen	10	(*)		
Seneca	12	(*)		
Taylor	10	(*)		

[1] The asterisk (*) indicates that the average number of aphids was more than 5 per cane on any 1 date.
[2] The asterisk (*) indicates that the average number of aphids was less than 5 per cane on any 1 date.
[3] Aphids absent.

In the field counts on the 25 varieties shown in table 4, *Amphorophora rubi* was not found on 4 and only a few aphids were found on 5 varieties. Seldom were more than three aphids found on any one cane of Antwerp at one time. Observations made in commercial plantings and experimental plots during 1935, 1936, and 1937 revealed that spread of mosaic was very slow in this variety, even in plantings where it was interplanted in the same row with mosaic Cuthbert plants. One count on Herbert, made August 2, showed an average of 5.5 aphids per cane in one of the plots. With few exceptions, the aphid populations on this variety were small. At times, Newburgh was found quite susceptible [8] to aphid infestation in the field even though greenhouse experiments and some field examinations indicated

[7] Under a cooperative agreement with the State Department of Agriculture aphid counts were made by one of its men while he was inspecting commercial fields for mosaic.

[8] ... August 10, an average of 34.6 aphids per cane were counted on Newburgh.

it to be partly resistant. Newburgh is classified in table 4 as susceptible in order to comply with the method of classification used.

RESISTANCE IN HYBRIDS

GREENHOUSE EXPERIMENTS

In 1936, greenhouse experiments with hybrids were limited to 50 selections from various crosses, including the resistant Lloyd George and several susceptible varieties. The results indicated that 6 of the hybrids were resistant to *Amphorophora rubi*, 13 partly resistant, and 31 susceptible. Of the hybrids in which resistance was indicated, one parent was the Lloyd George variety. A few hybrids between two susceptible parents were all susceptible.

In 1937, the 50 hybrids previously tested were retested, and in addition, all available inbred (self-pollinated) seedlings of Chief, Cuthbert, Latham, Lloyd George, and Newman were tested to obtain information as to the genetic constitution of these varieties in relation to aphid resistance. Fifty hybrids selected at random from crosses of Lloyd George and Chief also were tested. Crosses between these varieties were selected because of the marked aphid resistance in the Lloyd George and the marked aphid susceptibility in the Chief.

A summary of the results of the greenhouse experiments obtained in 1936 and 1937 is presented in table 5. Aphid populations on the susceptible seedlings ranged from 36 to more than 200 per cane tip. In a few cases, the aphid counts on plants of this group were less than 100, but in the majority of cases the counts exceeded 200. The relatively low counts on the partly resistant group contrasted sharply with the high aphid counts on the susceptible group. As the data of table 5 indicate, all inbred seedlings of the susceptible varieties, Chief, Cuthbert, Latham, and Newman showed definite susceptibility to *Amphorophora rubi*. Sixteen inbred seedlings of the resistant Lloyd George exhibited marked differences in susceptibility, only four being completely resistant and two partly resistant. The results of the tests of hybrids show definitely that Lloyd George transmits resistance in hybridization with susceptible varieties.

TABLE 5.—*Summary of aphid-resistance studies with red raspberry seedlings in the greenhouse during 1936 and 1937*

Seedlings tested (number)	Parentage	Susceptible [1]	Partly resistant [2]	Resistant [3]
5	Chief, self-pollinated	5		
6	Cuthbert, self-pollinated	6		
6	Latham, self-pollinated	6		
16	Lloyd George, self-pollinated	10	2	4
17	Newman, self-pollinated	17		
17	Lloyd George, open-pollinated	7	2	8
25	Chief × Lloyd George	16	1	8
25	Lloyd George × Chief	11		14
12	Cuthbert × Lloyd George	9	3	
16	Lloyd George × Cuthbert	9	4	3
4	Latham × Lloyd George	2	1	1
4	Lloyd George × Latham	3		1
1	King × Lloyd George		1	
1	Lloyd George × King		1	
2	Antwerp × Lloyd George	2		
2	Cuthbert × Latham	2		
2	Latham × Cuthbert	2		
1	Cuthbert × Marlboro	1		
1	Marlboro × Cuthbert	1		

[1] Average number of aphids more than 10 per cane tip.
[2] Average number of aphids less than 10 per cane tip.
[3] Aphids all dead.

FIELD OBSERVATIONS

Aphid counts were made in experimental plots only on those hybrids that had been selected for propagation because of superior fruit quality. All of these hybrids were included in the greenhouse investigations. The results of the field observations were in close agreement with those of the greenhouse experiments. Hybrids that harbored numerous aphids in the field showed susceptibility to aphids in the greenhouse and those that showed complete resistance in the greenhouse were consistently free of aphids in the field plots. Hybrids showing partial resistance in the greenhouse harbored few or no aphids in the field.

DISCUSSION

This investigation was undertaken to determine the practicability of attacking the red raspberry mosaic problem in western Washington by plant-breeding methods. In combating the mosaic disease, a variety that resists attack by mosaic vectors, and thereby escapes infection, will serve the same purpose as one that is resistant to the mosaic virus. The occurrence of resistance to *Amphorophora rubi* in important varieties such as Lloyd George therefore enhances their horticultural value. The fact that resistance is transmitted in hybridization offers a practical means of solving the mosaic problem in districts where *A. rubi* is the principal mosaic vector.

Throughout the investigation, there was a consistent agreement between the results of field observations and the results of greenhouse experiments. For example, the data in table 3, for field conditions, indicate the same relative susceptibility of varieties as the data of table 1, for greenhouse experiments.

It would be difficult to obtain an accurate comparison of varieties relative to aphid resistance by field observations alone, because of differences in aphid populations upon the same variety and rapid fluctuations caused by weather conditions. In comparison, greenhouse testing is more accurate because the aphids maintain steady populations and have an opportunity for maximum reproduction. Small aphid populations under greenhouse conditions should, therefore, be more significant than small populations under field conditions. On the other hand, greenhouse conditions may magnify the susceptibility of some varieties and hybrids that are relatively resistant under field conditions. The observation of the host-vector relationship in the field, therefore, is important as a supplement to greenhouse experiments.

The classification of varieties and hybrids into susceptible, partly resistant, and resistant groups is convenient for purposes of comparison but may not fully express the relative resistance of varieties except for those that are completely resistant. In the partly resistant group, Newburgh occasionally harbored larger numbers of *Amphorophora rubi* (footnote 8) than Herbert and Antwerp under similar conditions. Likewise, in the susceptible group, some varieties appeared to be more satisfactory than others as hosts for *A. rubi*. This suggests that a more detailed study of susceptibility might either indicate a more accurate grouping or eliminate grouping and show a gradation from complete resistance to high susceptibility. The experiments with hybrids do not clarify the situation. Aphid counts on 25 hybrids of

the cross Lloyd George × Chief (table 5) indicated only two classes. In other crosses, a wider variation in aphid populations was observed.

The results obtained in testing varieties and seedlings show that a variety may be completely resistant to *Amphorophora rubi* without being homozygous for resistance. Lloyd George is heterozygous because it transmits resistance to only a portion of its inbred and hybrid seedlings.

Although the number of seedlings tested was relatively small, it appears reasonably certain that Chief, Cuthbert, Latham, and Newman are homozygous for susceptibility. It remains to be determined whether any existing varieties are homozygous for resistance and whether homozygous resistant hybrids can be produced by hybridization.

SUMMARY

An investigation was undertaken during 1935, 1936, and 1937, to determine the resistance of named varieties and hybrids of red raspberry to the mosaic vector *Amphorophora rubi* Kalt. The study revealed that varieties of red raspberry possess various degrees of resistance to *A. rubi* in western Washington.

In the greenhouse *A. rubi* failed to reproduce and maintain its population on the varieties Indian Summer, Lloyd George, Pyne Imperial, and Pyne Royal. Reproduction was slow and the population remained small on the varieties Antwerp, Herbert, Marcy, and Newburgh. Aphids fed and reproduced abundantly on 19 varieties; namely, Cayuga, Chief, Cuthbert, June, Katherine, King, Latham, Laxton Bountiful, Laxton Renown, Laxton Reward, Marlboro, Newman, Preussen, Ranere (St. Regis), Red Cross, Rote Riesen, Seneca, Taylor, and Viking.

In experimental plots and commercial plantings *A. rubi* was found not to feed on those varieties that showed resistance to the aphid in the greenhouse. Populations were small on those showing partial resistance and comparatively large on the susceptible varieties.

Greenhouse experiments and field observations with red raspberry seedlings showed resistance to *A. rubi* to be inherited and transmitted when a resistant variety was crossed with a susceptible one. The variety Lloyd George was found to be heterozygous for resistance.

Greenhouse testing is a convenient and rapid method for determining the relative resistance of varieties and hybrids of red raspberry to *A. rubi*.

LITERATURE CITED

(1) Bennett, C. W.
1932. Further observations and experiments with mosaic diseases of raspberries, blackberries, and dewberries. Mich. Agr. Expt. Sta. Tech. Bull. 125, 32 pp., illus.

(2) Cooley, L. M.
1936. Wild brambles in relation to spread of virus diseases in cultivated black raspberries. N. Y. State Agr. Expt. Sta. Bull. 665, 15 pp.

(3) Jones, Leon K., and Baur, Karl E.
1936. Mosaic and related diseases of raspberries in Washington. Wash. Agr. Expt. Sta. Bull. 324, 19 pp., illus.

(4) Knowlton, G. F., and Allen, M. W.
1937. Bramble fruit aphids. Ann. Amer. Ent. Soc. 30: 309–316, illus.

(5) RANKIN, W. HOWARD.
1927. MOSAIC OF RASPBERRIES. N. Y. State Agr. Expt. Sta. Bull. 543, 60 pp., illus.
(6) SCHWARTZE, C. D., and HUBER, GLENN A.
1937. APHIS RESISTANCE IN BREEDING MOSAIC-ESCAPING RED RASPBERRIES. Science 86 (2224): 158–159.
(7) SLATE, GEORGE L., and RANKIN, W. HOWARD.
1933. RASPBERRY GROWING IN NEW YORK STATE: CULTURAL PRACTICES AND DISEASE CONTROL. N. Y. State Agr. Expt. Sta. Bull. 625, 62 pp., illus.
(8) WINTER, J. D.
1929. A PRELIMINARY ACCOUNT OF THE RASPBERRY APHIDS. Minn. Agr. Expt. Sta. Tech. Bull. 61, 30 pp.

U. S. GOVERNMENT PRINTING OFFICE: 1938

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 57 WASHINGTON, D. C., NOVEMBER 1, 1938 No. 9

THE CAROTENE CONTENT OF MARKET HAYS AND CORN SILAGE[1]

By HERBERT G. WISEMAN, *assistant chemist*, EDWARD A. KANE, *assistant chemist*, LEO A. SHINN, *assistant chemist*, and C. A. CARY, *senior chemist, Division of Nutrition and Physiology, Bureau of Dairy Industry, United States Department of Agriculture*[2]

INTRODUCTION

The importance of vitamin A in cattle rations has been recognized for several years; and as more definite knowledge has accumulated regarding the quantity of vitamin A required by dairy cattle, more exact information about the vitamin A potency of farm feeds has become desirable and usable in feeding these animals. Although a number of conditions besides the quantity of vitamin A or its precursors may affect the vitamin A potency of a feed, the determination of the quantity of its precursors in feeds of plant origin is, at the present time, by far the best available measure of their vitamin A value.

In connection with the work done in the laboratory of the Division of Nutrition and Physiology at Beltsville, Md., to determine the vitamin A requirements of cattle, all the market hays that have been fed and many samples of the corn silage fed, have been analyzed for carotene. In addition, the hays have been graded by representatives of the Division of Hay, Feed, and Seed of the Bureau of Agricultural Economics.

There have thus been collected considerable data on the carotene content of the various market grades of hay—particularly of alfalfa and timothy hays—and the relation between certain physical characteristics of these hays and their carotene content, which might be of use in determining to what extent hays of suitable vitamin A potency may be selected by ordinary inspection or grading.

In analyzing the samples of corn silage, it was early noted that corn silages differ greatly in their vitamin A value and that while some are a rich source of vitamin A others contain too little to be used as the principal source of vitamin A in cattle rations. Work was therefore done to determine some of the conditions that influence the vitamin A value of corn silage.

The routine analytical methods that have been used in the Beltsville laboratory, and also in other laboratories, for the determination of carotene in cattle feeds have depended upon some modification of the well-known Willstätter and Stoll procedure for separating chlorophyll and xanthophyll from carotene. More information is needed regarding the accuracy of these methods when applied to hays and silages,

[1] Received for publication March 17, 1938; issued November 1938.

[2] The writers are indebted to J. T. Scanlon and Mrs. A. P. Bradshaw of the Color and Farm Waste Laboratory of the Bureau of Chemistry and Soils, U. S. Department of Agriculture, for assistance during several years in the use of the spectrophotometer in their laboratory; to K. S. Gibson of the Division of Colorimetry and Spectrophotometry of the National Bureau of Standards, U. S. Department of Commerce, for advice on a number of occasions, for the use of standardized glasses, and for the readings made in his laboratory in connection with some of the spectral absorption curves; and to W. A. Wheeler, chief, and members of his staff—especially C. F. Welsh and W. H. Hosterman of the Division of Hay, Feed, and Seed, Bureau of Agricultural Economics, U. S. Department of Agriculture for the grading of the hays used in this work.

and possibly also when they are used in analyzing the freshly cut green plant. Considerable work has also been done in various laboratories by using modifications of the Willstätter and Stoll procedure to separate the cholorophyll and xanthophyll from the carotene, and it seemed desirable to know to what extent the results from these different laboratories are actually comparable.

In this paper, the writers (1) describe the procedure used at present in the Beltsville laboratory to determine the carotene content of cattle feeds; (2) consider the results they have obtained on the accuracy of this procedure and the comparability of such methods when applied to hays and corn silage; and (3) present the data they have obtained on the carotene content of market hays and corn silage, together with a consideration of the practical usefulness of these results in feeding dairy cattle. These topics are taken up practically in the order mentioned; but, before proceeding with this outline, the writers discuss briefly some of the more closely related investigations that have led up to some phases of the work reported on in this paper. No attempt is made, however, to review all the literature on the vitamin A value of roughages, much of which is doubtless familiar to most workers who are interested in the subject.

REVIEW OF PREVIOUS, RELATED INVESTIGATIONS

It has been 30 years since work was begun in Wisconsin on cattle that were observed to be suffering from symptoms that are now known to result (*10*, *25*),[3] in part at least, from a deficiency of vitamin A in the ration. This factor had not been distinguished as a separate dietary essential; and a long period intervened before cod-liver oil was found to ameliorate these symptoms in cattle (*10*), and before it was shown that for the normal nutrition of calves the diet must contain some source of vitamin A (*14*). It is now well known (*2*, *3*, *11*, *27*, *28*, *29*) that vitamin A or its precursors may be so deficient in ordinary cattle rations as to lead to disaster in reproduction, to difficulty in rearing the young, or to a deficiency of vitamin A in the milk. Also, several laboratories (*9*, *29*, *32*) have reported work on the quantity of vitamin A required by cattle to bring about certain physiological responses.

Formerly, alfalfa hay was generally regarded as an excellent source of vitamin A; but Meigs and Converse (*27*, *28*, *29*) found that the failure in reproduction—as well as the deficiency of vitamin A in the milk—which they observed when the cows were fed a poor quality of alfalfa hay continuously as the main source of vitamin A, was due to the low vitamin A value of the ration they were feeding. They also encountered similar results when the poorer grades of timothy hay were similarly fed.

Previous work in the Beltsville laboratory (*12*) with rats had shown that the carotene in alfalfa hay accounts completely for its vitamin A potency. Under suitable conditions, all the known precursors of vitamin A in plant materials occur in the carotene fraction, when analytical methods based on the Willstätter and Stoll procedure are used for the separation of the plant pigments. So little of these precursors of vitamin A exist in the grains used in dairy rations that, even with a liberal amount of yellow corn in the ration, a cow usually

[3] Italic numbers in parentheses refer to Literature Cited, p. 667.

must depend on the roughages in the ration for well over 90 percent of her vitamin A requirements.

Beta-carotene is by far the most important precursor of vitamin A in hays and corn silage. It appears to be practically the only one in alfalfa (*41*) and the grasses (*15, 22, 31*). Little is known regarding the relative quantities of this pigment that cattle assimilate from various plant sources and dietary mixtures. Evidence has been presented to show that soybeans, for instance, may possibly affect the utilization of carotene in the ration, at least insofar as the effect of this pigment upon the vitamin A content of butter is concerned (*13*). Nevertheless, it appears that the quantity of beta-carotene in hays and corn silage is by far the best available measure of the relative value of these feeds as a source of vitamin A.

The other known precursors of vitamin A in plant materials are much less efficiently utilized than beta-carotene (*5, 21*); and the amounts in which they occur in most cattle feeds are relatively negligible. The carrot is a notable exception, the carotene from its root being a mixture of about 10 to 20 percent of alpha-carotene, traces of gamma-carotene, and 80 to 90 percent of beta-carotene (*15, 22, 41*). Although cryptoxanthine is said to account for most of the vitamin A potency of the yellow corn kernel, the proportion of the vitamin A in cattle rations that is due to this pigment is quite small. The proportion of the vitamin A potency of corn silage that is due to the cryptoxanthine in the corn kernel is also negligible.

The accuracy of the determination of the vitamin A potency of alfalfa or timothy hay or of corn silage depends, therefore, upon the accuracy with which the quantity of beta-carotene in these feeds can be estimated. In most of the analyses at hand, it has been assumed not only that practically all of this pigment in these feeds occurs in the final carotene fraction that is used to estimate it either colorimetrically or spectrophotometrically, but also that no other pigment that would affect these measurements is present in this carotene fraction. This latter assumption may be very nearly true for such freshly cut, green plant materials as the grasses and alfalfa (*46*); but carotene fractions from a sample of alfalfa hay that were tested biologically for vitamin A potency in the Beltsville laboratory were found to be somewhat less active than the amounts of beta-carotene estimated on the basis of this assumption to be present in them. The difference in the growth of the rats used in this experiment, however, amounted to only about 20 percent (*12*).

Further, it is known that in plants cut for hay, there is a very rapid destruction of the carotene during the curing process (*36, 37*), and that this destruction continues, although at a somewhat slower pace, during subsequent storage (*8, 46*). Intermediate colored decomposition products are known to be formed in the oxidation of carotene (*18, 26*); and, according to Kuhn and Brockmann (*18*), not all of such products are readily removed from the carotenes by methods of analysis based on the Willstätter and Stoll procedure for the removal of the chlorophylls and xanthophylls. One might therefore suspect the presence of such pigmented impurities in the hays even if they did not occur in the freshly cut plant materials.

It is further known that the carotenes to some extent, but more especially the xanthophylls, are attacked by acids. Kuhn (*18*) and

his collaborators found that the xanthophylls thus yield colored decomposition products that may also contaminate carotene fractions prepared by the Willstätter and Stoll procedure (*18*, *23*). It seems, therefore, that the possibility of the occurrence of such xanthophyll decomposition products in the carotene fractions, especially from the silages, should also be considered.

Several biologically active oxidation products of carotene have been prepared (*4*, *19*, *20*); but the only naturally occurring products of this sort that have been tested biologically for vitamin A activity, apparently, are those that have been tested in the Beltsville laboratory, and these have been found to be inactive.

Kuhn and Brockmann (*18*) describe adsorption methods for the separation of carotene from its colored oxidation products and from the products formed from xanthophyll by the action of acids. No evidence is presented by these workers, however, to show that the carotene itself may not be altered in the procedure.

ANALYTICAL PROCEDURE FOR THE DETERMINATION OF CAROTENE IN FARM FEEDS

DESCRIPTION OF ROUTINE ANALYTICAL METHODS

Two analytical methods (A and B) have been used for the routine determination of carotene in the work reported in this paper. They differ only in the way the samples of feed were ground and the carotene extracted from them. Method B is described here as it is used in analyzing hays and such succulent materials as the silages and the freshly cut plant. Both methods A and B, like most methods in practical use, depend upon a modification of the Willstätter and Stoll procedure for separating the carotene from the other plant pigments.

METHOD A

Method A is usable with materials that may be ground readily and completely in a Wiley mill. It is particularly useful with hays. With baled hays the bales are bored repeatedly with a 2-inch drill. The drillings are ground through the medium sieve (1-mm holes) and then through the finest sieve (0.5-mm holes) in a Wiley mill. Aliquot samples are taken for analysis. Absolute ethanol, 15 ml for a 6-g sample, is added; and, after standing 1 hour or more (overnight), the mixtures are extracted six times or more by shaking for 15 minutes with low-boiling (30° to 60° C.) petroleum ether (20-ml portions for a 6-g sample). A sintered glass funnel is used in removing the extract. The combined extract is concentrated in vacuo to 75 to 100 ml; 20 ml of water is added; and after the solution is shaken and mixed by swirling gently, the alcohol-water layer is discarded. The chlorophyll and about 95 percent of the xanthophyll are removed from the carotene by two treatments of the ligroin fraction with a saturated potassium hydroxide-methanol (10 ml each time) solution. The mixture is shaken 15 minutes during each treatment; the alcoholic layer is then diluted with 1 ml of water, the shaking continued for 2 to 5 minutes more, and the diluted alcoholic potash layer is discarded. The petroleum ether fraction is then washed free of xanthophyll by shaking it for 2 minutes repeatedly with 25-ml portions of methanol, 92 percent by volume. The methanol used in the final washing should be colorless.

The carotene fraction is then concentrated in vacuo and brought to volume with a 1:1 mixture of ethanol and higher boiling (about 90° C.) petroleum ether. The ethanol retards the oxidation of the carotene.

Method B

Method B is suitable for the determination of carotene in all sorts of material; but it has been found especially useful with dry materials that are difficult to grind satisfactorily in a Wiley mill, and for succulent substances such as the freshly cut plant, silages, etc. In this method the sample is ground to a fine powder while immersed in concentrated ethanol (approximately 98 percent by volume after allowance is made for the moisture from the sample).

Dry materials are first ground through the medium sieve (1-mm holes) in a Wiley mill, and then with alcohol in the ball mill.

The corn plant, when analyzed, is first put through a meat grinder and the ground material is collected directly in absolute ethanol. This mixture is allowed to stand overnight in an ice box; the aqueous-alcohol fraction is then removed and the residue ground in a fresh lot of absolute ethanol in the ball mill. The silages were formerly handled in the same way as the freshly cut corn plant; but later it was found that corn silage need not be ground in a meat grinder before it is immersed in the alcohol.

With freshly cut plant materials such as grasses, alfalfa, etc., absolute ethanol is taken into the field and cooled with solid carbon dioxide to between −60° and −70° C. The sample when cut is immediately frozen in this mixture and the brittle material broken up with a pestle. After standing overnight in an ice box, the sample is handled in the same way as the silages.

With succulent materials enough alcohol is used in the preliminary alcoholic treatments to give an alcohol-water mixture containing close to 90 percent or more of alcohol. This alcohol-water mixture is poured off quite completely; a Büchner funnel is used, if necessary, to recover the residue. The second alcoholic extract, which contains almost all the carotene, may be filtered onto a Büchner funnel, the residue being washed thoroughly with additional alcohol; or the residue simply may be allowed to settle out, the water from the original sample being allowed for in calculating the total volume of this extract.

The two alcoholic extracts obtained in these extractions are aliquoted separately and the aliquots combined. Water is added to reduce the alcoholic concentration to 85 percent; the carotene is extracted out of this diluted alcoholic solution by shaking twice with the low-boiling petroleum ether; and the combined carotene extract is then saponified, washed with 92-percent methanol and brought to volume as in method A.

In the ball mill, 1,600 ml of absolute ethanol have been used with 100-g samples of the hays or freshly cut grasses or freshly cut alfalfa and with 300-g samples of corn silage or of the freshly cut corn plant. In the preliminary alcoholic treatments, with the samples from the corn plant and corn silage 3 liters of absolute ethanol were used and 1 liter with the grasses and freshly cut alfalfa. All of the petroleum ether used in this work was treated with concentrated sulphuric acid,

washed with water, and then treated with potassium permanganate to remove peroxides.

All the samples reported in this paper have been read with a spectrophotometer—many at wave length 436 m μ. The carotene has been calculated on the basis of $E_{1cm}^{1\mu g \text{ per ml}} = 0.195$ at wave length 436 mμ. Frequently the samples have also been read at other wave lengths to obtain light on the character of the pigmented material in them. A colorimeter equipped with a mercury light and a light filter that transmits the 436 or 436 and 405 bands of light, when used with a potassium dichromate standard, gives relative results that are practically identical with those obtained with the spectrophotometer at wave length 436 mμ. The photoelectric machine described by Brice (*1*) has also been found to be very satisfactory when adequately calibrated and used with a light filter. The Corning Glass Works filter No. 556 has been used.[4] [5]

LOSS OF CAROTENE IN THE GRINDING AND EXTRACTION OF HAYS FOR CAROTENE ANALYSIS

Previous work in the Beltsville laboratory (*12*) has shown that when finely pulverized hay is extracted by method A, no biologically detectable amount of carotene is destroyed, and only a trace of vitamin A potency remains in the extracted residue. In that experiment this trace unquestionably amounted to only a small fraction of 10 percent of the total carotene originally in the powdered hay. The data in table 1 indicate further that it is unlikely that any considerable loss of carotene occurs during the grinding of a hay to a fine powder while it is immersed in alcohol in a ball mill as in method B. The results that are summarized in table 2 show, also, that the extraction of carotene from hays by method B is practically as complete as by method A, and that the method of grinding used in method A, is, therefore, very nearly as satisfactory as that in method B.

TABLE 1.—*Effect of the duration of grinding hay samples, while immersed in alcohol in a ball mill, on the carotene found*

Alfalfa hay sample No.[1]	Carotene per kilogram found when hay sample was ground for—				
	2 hours	3 hours	4 hours	5 hours	17 hours
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
1	53.9	53.9	54.3	54.3	53.4
2	----	81.0	----	79.0	79.6

[1] The spectral absorptive properties of the different aliquots of each sample of hay were the same regardless of the length of time of grinding.

[4] The samples were read visually on a Bausch & Lomb Universal spectrophotometer in the color laboratory of the Bureau of Chemistry and Soils at Arlington, Va. Many of the samples were read at Beltsville by either the colorimetric or the photoelectric procedure, described here, before and after they were read at Arlington. There was no apparent loss of carotene in taking the samples to Arlington. Ten or more readings were made at each wave length of light used in this work, the cups being switched.

[5] Some modifications in procedure have been made from time to time, but none of these has affected materially the results included in this paper.

TABLE 2.—*Comparison of results for carotene as obtained by methods A and B*

Samples of hay analyzed (number)	Method of analysis	Average carotene content per kilogram
		Milligrams
Average of 4 samples	Method B	28.8
	Method B, with subsequent extraction of the powdered residue with ligroin as in method A.	29.6
Average of 9 samples	Method A	17.2
	Method A, with subsequent extraction of the powdered residue with ligroin as in method A.	17.4
Average of 6 samples	Method A	15.5
	Method B	16.1

Efforts to obtain additional carotene from hay residues, after they have been ground and the carotene has been extracted according to method A, by treating them again with alcohol and by repeated extractions with ligroin have yielded further traces of pigment which at times may have been equivalent to as much as 3 or 4 percent of the total amount of carotene found in the hay. This small amount of carotene has been disregarded in the results presented in this paper.

The results cited in tables 1 and 2 favor the conclusion that very little, if any, destruction of carotene occurs in the grinding and extraction procedure by either method and that only a negligible amount of this pigment remains in the hay residues after extraction.

When method B is used to determine the amount of carotene in silages and succulent plant materials, the extraction of this pigment is undoubtedly as complete as with the hays, as the final conditions under which the extraction is carried out are the same. Further, in the analysis of freshly cut plant materials which are immediately immersed in alcohol at −60° to −70° C., the destruction of carotene must certainly be very small. Even when the sample is not immediately frozen in alcohol but is simply ground through a meat grinder, collected in the alcohol at room temperature, and analyzed as described in method B, the loss of carotene is still negligible. For example, when three lots of freshly cut, green alfalfa were analyzed by these two methods, the average carotene content per kilogram of dry weight was 239 and 214 mg, respectively. It would appear, therefore, that insofar as the grinding and extraction procedure is concerned, method B is as satisfactory for determining the carotene content of freshly cut plant materials and silages as it is for hays.

LOSS OF CAROTENE IN THE SAPONIFICATION OF THE CHLOROPHYLL AND IN THE SUBSEQUENT WASHING OF THE LIGROIN SOLUTION WITH METHANOL, AND THE COMPLETENESS OF REMOVAL OF THE XANTHOPHYLL AND CHLOROPHYLL

The data in table 3 show that when a solution of pure beta-carotene in low-boiling ligroin is treated with a saturated solution of potassium hydroxide in methanol to remove the chlorophyll, as described in method A, the loss of carotene in the two saponifications that are necessary for this purpose is less than 1 percent. There was no evidence that the carotene was altered chemically by these treatments. Along with the chlorophyll, about 93 percent of the xanthophyll was also separated from the carotene.

TABLE 3.—*The loss of beta-carotene and the removal of leaf xanthophyll from the carotene when solutions of these pigments in low-boiling petroleum ether are treated with a saturated solution of potassium hydroxide in methanol for the removal of chlorophyll from carotene as described in method A*

Pigment originally in ligroin solution	Quantity initially present	Initial volume	Saponification treatments	Dilution of alcohol before separation	Quantity finally in petroleum ether layer	Removed from petroleum ether layer
	Micrograms	*Milliliters*	*Number*	*Percent*	*Micrograms*	*Percent*
Beta-carotene	63.15	[1] 50	[2] 8	92	63.10	0.1
				90	62.18	1.5
Xanthophyll	1,280.00	[3] 100	2	92	84.00	93.4
				92	78.00	93.9

[1] The volume was made to 50 ml at the beginning of each saponification.
[2] The spectral absorption of the initial and final carotene solutions was determined at wave lengths 430 mμ, 450 mμ, and 480 mμ. The relation between the absorptions at these wave lengths was not changed as a result of these 8 saponifications.
[3] These solutions were saturated. The leaf xanthophyll that was used melted at 177° to 178° C. (uncorrected).

The results in table 4, with leaf xanthophyll in low-boiling ligroin, indicate that over 99 percent of it is removed from this solution by five washings with 92-percent methanol as described in method A. The trace of pigment that appeared to remain in the petroleum ether—possibly about 0.5 microgram per milliliter [6]—was not removed by further washing. The quantity of xanthophyll in these samples was approximately four times as much as could be present in a carotene analysis after removal of the chlorophyll as described above. The results show a more complete extraction of the xanthophyll by the 92-percent methanol than was observed by Miller (*30*).

TABLE 4.—*Completeness of removal of xanthophyll from its solution in low-boiling petroleum ether by washing this solution with 92-percent methanol as described in method B* [1]

Sample No.	Xanthophyll initially in sample	Volume of petroleum ether layer		Washings	Xanthophyll finally in petroleum ether solution	Xanthophyll concentration per milliliter in final volume	Xanthophyll removed
		Initial	Final				
	Micrograms	*Milliliters*	*Milliliters*	*Number*	*Micrograms*	*Micrograms*	*Percent*
1	360	75	58	5	3.0	0.05	99.2
		75	54	6	3.1	.06	99.1
2	360	75	54	5	2.7	.05	99.2
		75	49	6	2.5	.05	99.3

[1] The final extract was colorless in all cases.

The data in table 5 show the amount of pure carotene that may be lost from the ligroin phase as a result of washing its solution in low-boiling ligroin with diluted methanol to remove the xanthophyll. The loss with 90-percent methanol and 10 washings or with 92-percent methanol and 5 or 6 washings, is slightly over 1 percent.

[6] This was estimated photoelectrically from a very slight absorption of light by this solution.

TABLE 5.—*Loss of beta-carotene as a result of washing its solution in low-boiling ligroin with diluted methanol for the removal of the xanthophyll as described in method A*

Sample No.	Pigments initially in ligroin layer		Initial volume of ligroin layer	Washings	Temperature	Concentration of methanol	Pigment calculated as carotene left in ligroin layer	Recovery of carotene, based on carotene in original solution
	Kind [1]	Quantity						
		Micrograms	*Milliliters*	*Number*	*°C.*	*Percent*	*Micrograms*	*Percent*
K-1, I-S	Beta-carotene	52.50	25	[2] 10		92	[3] 51.30	[3] 97.7
K-2, I-S	do	52.50	25	[2] 10		90	[3] 52.00	[3] 99.0
W-1, I-S	do	63.15	[4] 50	[2] 10		90	62.38	98.8
W-2 and 3	do	575.00	100	5	28	92	572.50	99.6
W-4 and 5	do	575.00	100	5	15–17	92	568.00	98.8
W-6 and 7	do	531.00	100	6		92	522.00	98.3
W-8 and 9	do	531.00	100	6		92	527.00	[5] 99.2
	Leaf xanthophyll	1,045.00						

[1] The melting point of the leaf xanthophyll was 177° to 178° C. (uncorrected). The beta-carotene extracts in the I-S samples were fractions of the second international standard carotene.

[2] The initial and final solutions were read spectrophotometrically (visual method) at wave length 450 mμ, 480 mμ, and at either 430 mμ or 436 mμ. There was no evidence of any change in the character of the pigment as a result of these washings.

[3] There was some mechanical loss with this sample. The loss was larger with K-1 than with K-2, but an examination of the methanol layer by driving the ligroin dissolved in it out of solution by the addition of water showed some color in this layer in the case of the 92-percent methanol.

[4] This sample was made to 50 ml before each extraction.

[5] The final volume of the ligroin solution was 80 ml with each sample. If the concentration of the xanthophyll left in the ligroin phase is the same as shown in table 3, the unextracted xanthophyll would be 4+ μg, and the recovery of the carotene corrected for this xanthophyll would be 98.5 percent.

The results in table 6 indicate the extent of the loss of the carotene that may occur in the routine analysis of alfalfa hay when the procedure described in method A is used to remove the chlorophyll and xanthophyll. The loss in washing with 92-percent methanol is not detectably greater than it was with pure beta-carotene itself; but in saponifying to remove the chlorophyll this loss is apparently somewhat greater—being about 2 percent for two treatments of this nature. In another effort to get at the magnitude of these losses the final carotene fraction from a sample of hay was divided into two aliquots. One was saponified four additional times as described in method A and then washed seven additional times with 92-percent methanol. The decrease in absorption that could be attributed to a loss of carotene was between 1.5 and 3.4 percent; and, as will be shown presently, these "carotene" fractions from hays and silages contain pigments other than carotene that may account for a considerable portion of this loss.

TABLE 6.—*Loss of carotene from alfafa hay in separating the carotene from chlorophyll and xanthophyll*

Aliquots No.[1]	Saponifications	Washings with 92-percent methanol	Carotene found per gram of sample	Additional loss as compared to aliquots 1 and 2
	Number	*Number*	*Micrograms*	*Percent*
1 and 2	4	4	22.8	
3 and 4	8	4	21.9	3.9
5 and 6	4	8	22.7	.4

[1] 1 hay sample was ground and extracted, and aliquots of the extract were analyzed as indicated in the table.

After a ligroin solution of chlorophyll was saponified and then washed with methanol as decribed in method A, no visible color remained in the ligroin phase. Apparently this procedure completely removes the chlorophyll.

To sum up, the methods described above for the determination of carotene in feeds may fail to extract a small amount of the carotene (at most 3 or 4 percent); and an additional loss of possibly 2 or 3 percent ordinarily may occur in separating it from the chlorophyll and xanthophyll. The greater part of these losses of carotene may be avoided by re-extraction of the feeds and by extracting back in case of the saponifications and of the washings with 92-percent methanol. These losses have been disregarded in the data presented herein.

PIGMENTED IMPURITIES IN CAROTENE FRACTION FROM HAYS AND CORN SILAGE AFTER SAPONIFICATION AND SUBSEQUENT WASHING OF THE LIGROIN SOLUTION WITH METHANOL

The largest error in the estimation of the carotene in hays and corn silage by analytical methods in which the separation of the pigments is carried out solely by saponification, and subsequent washing of the ligroin solution of the carotene with 90- or 92-percent methanol, is apparently associated with the occurrence of colored impurities in the final carotene fraction that is used in the colorimetric or spectrophotometric measurements. The presence of such pigments is indicated by the biological activity of these carotene fractions, by their spectral absorption, and by their behavior when filtered through absorption columns. This evidence will be considered here.

The work of Hartman and associates (*12*) shows that when carotene extracts, prepared from pulverized alfalfa hay according to method A, are fed to rats that have been depleted of vitamen A, the resulting rate of growth is about 20 percent less than when the rats are fed doses of beta-carotene that were equivalent to the extracts in adsorption at wave length 436 mμ.

The data in tables 7 and 8 permit a comparison at certain wave lengths of the spectral absorption of various feeding materials with that of the alpha- and beta-carotene and leaf xanthophyll.

TABLE 7.—*Relative spectral absorption of xanthophyll, alpha-, and beta-carotene at various wave lengths of light expressed in percentage of the absorption at wave length 450 mμ*

Pigment	Relative spectral absorption at wave lengths of—						
	430 mμ	436 mμ	440 mμ	450 mμ	460 mμ	470 mμ	480 mμ
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Beta-carotene [1]	72.0	77.0	81.9	100	92.2	83.7	89.4
Alpha-carotene [2]	70.8	83.0	92.5	100	77.8	88.0	88.9
Leaf xanthophyll [3]	73.9	86.6	98.1	100	79.0	91.2	81.8
Second internation standard carotene [4]	72.3	------	------	100	93.1	82.7	[5] 87.3

[1] Data are averages calculated from charts by Miller (*31*), Smith (*40*), and McNicholas (*26*).
[2] Data are averages calculated from charts by Miller (*31*), and Smith (*40*).
[3] Data are averages calculated from charts by Miller (*31*), and McNicholas (*26*).
[4] Data were determined by the laboratory of the Bureau of Dairy Industry, by using spectrophotometer at color laboratory of Bureau of Chemistry and Soils.
[5] This low reading appears to be due to an error in the wave-length reading at 480 mμ, as results in accord with this assumption were obtained with color glasses standardized at the National Bureau of Standards, Washington, D. C.

TABLE 8.—*Relative spectral absorption of carotene extracts from various cattle feeds, expressed in percentage of the absorption at wave length 450 mμ*

Cattle feeds, from which carotene extracts were prepared [1]	Lots averaged	Spectral absorption at wave length of—		
		430 mμ	450 mμ	480 mμ
	Number	*Percent*	*Percent*	*Percent*
Second international standard carotene		72.3	100	87.3
Freshly cut green alfalfa		73.1	100	87.4
Artificially dried alfalfa meal	3	73.6	100	87.7
Alfalfa hay:				
U. S. No. 1	12	77.1	100	85.5
U. S. No. 3	4	79.8	100	82.2
Timothy hay:				
U. S. No. 1	6	78.5	100	84.7
U. S. No. 3	12	83.1	100	83.0
Corn plant chopped for ensiling		73.8	100	84.5
Corn silage	12	80.2	100	84.0

[1] Extracts prepared according to methods A and B described in the text. The hays were graded by representatives of the Bureau of Agricultural Economics.

If one allows for the variations that may result in such data from the use of different machines, different methods of reading, etc., it is obvious that the data for the carotene extracts from the freshly cut alfalfa and artificially dried alfalfa leaf meals resemble those for beta-carotene as nearly as can be expected; but that with the extracts from the hays and corn silage this is not true. With these extracts the relative absorption, as expressed in tables 7 and 8, is too high at wave length 430 mμ and too low at 480 mμ to correspond with either alpha- or beta-carotene and is not of a nature to be accounted for by assuming the presence of unremoved xanthophyll in the carotene. The discrepancy between the absorption of these extracts and that of the pure pigments is greater with extracts from the poorer quality hays.

The data in tables 9 and 10 show the results obtained when the carotene extracts from the hays and corn silage are filtered through adsorption columns.

TABLE 9.—*Effect of filtering the carotene extracts, prepared from hays and corn silage, according to methods A and B, through adsorption columns prepared from magnesium oxide*

Sample of feed analyzed	Wave length of light	Carotene extract not filtered through adsorption column		Carotene extract filtered through adsorption column				Pigmented impurity in unfiltered carotene fraction
				Carotene fraction from column		Impurity from column		
		Spectral absorption ratio [1]	Carotene, per gram of feed	Spectral absorption ratio [1]	Carotene per gram of feed	Spectral absorption ratio [1]	Equivalence in carotene, per gram	
	Millimicrons	*Percent*	*Micrograms*	*Percent*	*Micrograms*	*Percent*	*Micrograms*	*Percent*
Alfalfa hay, lot 85, U. S. No. 1	430	75.9		73.7		97.8		
	450	100.0	52.1	100.0	46.2	100.0	5.4	11
	480	85.2		87.6		68.2		
Alfalfa hay, lot 87, U. S. No. 3	430	79.7		[2] 74.4		95.5		[2] 32
	450	100.0	3.2	100.0	2.4	100.0	.77	
	480	85.1		[2] 86.2		74.2		
Timothy hay, lot 73, U. S. No. 1	430	75.3		73.6		93.0		
	450	100.0	32.2	100.0	28.7	100.0	3.6	
	480	84.6		87.2		72.7		11
Timothy hay, lot 79 U. S. No. 3	430	79.6		[3] 75.3		97.0		
	450	100.0	5.43	100.0	4.42	100.0	.94	[3] 30
	480	83.4		[3] 85.9		74.0		
Corn silage	430	76.7		72.8		86.7		
	450	100.0	[4] 42.4	100.0	33.0	100.0	9.6	23
	480	85.0		87.5		74.6		

[1] Expressed in percentage of the absorption at 450μ.

[2] The adsorption filtration apparently left about 7 percent of unremoved impurity still in the carotene fraction, making a total of about 32 percent of impurity in the original unfiltered sample.

[3] The adsorption filtration apparently left about 11 percent of unremoved impurity still in the carotene fraction, making a total of about 30 percent of impurity in the original unfiltered sample.

[4] Dry weight.

TABLE 10.—*Recovery of pigments in carotene extracts, prepared from hays and corn silage, when these extracts are filtered through magnesium oxide adsorption columns*

Sample of feed analyzed	Wave length of light	Absorbency readings under comparable conditions [1]			
		For carotene extracts filtered through adsorption columns			For unfiltered carotene extracts
		Carotene fraction	Pigmented impurity	Total	
	Millimicrons				
Alfalfa hay, lot 85, U. S. No. 1 [2]	430	0.813	0.127	0.940	0.940
	450	1.104	.129	1.233	1.239
	480	.969	.087	1.056	1.050
Alfalfa hay, lot 87, U. S. No. 3	430	.777	.317	1.094	1.105
	450	1.035	.332	1.367	1.381
	480	.905	.246	1.151	1.175
Timothy hay, lot 73, U. S. No. 1 [3]	430	.761	.122	.883	.872
	450	1.034	.131	1.165	1.159
	480	.903	.095	.998	.980
Timothy hay, lot 79, U. S. No. 3	430	.599	.164	.763	.778
	450	.796	.169	.965	.978
	480	.684	.125	.809	.816
Corn silage	430	.480	.178	.658	.658
	450	.657	.208	.865	.873
	480	.574	.162	.736	.723

[1] Samples comparable in size, final volume, and thickness of absorbing layer.
[2] 3 aliquots were filtered and the results averaged.
[3] 2 aliquots were filtered and the results averaged.

These adsorption columns were made from magnesium oxide (Merck). This material was activated by moistening, heating on a steam bath for 2 hours or more to bring it almost to dryness, and then heating for 1 hour in a muffle furnace at 200° C. It was ground through a 200-mesh sieve, and diluted with the same brand of siliceous earth used by Strain (*42*) to increase the rate of filtration.

The adsorption filtrations were carried out in an atmosphere of nitrogen (purified over copper) in an apparatus that was so arranged that all liquids could be boiled in a reduced atmosphere of nitrogen before coming into contact with the column. The column itself was only very mildly activated. This condition was selected after considerable experimentation, which indicated that with more active columns of this type there is a very considerable destruction of the pigments, including even the carotene. It has since been found desirable to cool these columns with ice water during filtration, especially during warm weather.

In these adsorption filtrations the pigments from the carotene extracts from the hays and corn silage separated readily into two fractions—one (the carotene) which was easily washed through the column with ligroin (boiling at 35° to 60° or 90° to 100° C.), and a second (the colored impurity) which adsorbed more strongly on the column and was eluted later with ligroin containing a small amount of ethanol. As shown in table 9, the former fraction when derived from corn silage or from hays of good quality, resembles beta-carotene very closely in spectral absorption; whereas the spectral absorption of the more strongly adsorbed fraction was such as to account for the difference, noted previously, between that of beta-carotene and the original unfiltered extract. With the poorer quality hays, it is evident that not

all of this colored impurity was removed from the carotene in the one filtration that was used.

Carotene extracts, prepared according to method B from freshly cut plant materials (alfalfa, Kentucky bluegrass, and corn plant) have likewise been filtered through magnesium oxide-adsorption columns. Although the spectral absorption of the pigments in the alfalfa and bluegrass extracts, both before and after the adsorption filtration, resembled that of beta-carotene closely and within the limit of the experimental error of the writers' methods of measurement, a small amount of pigmented impurity was removed by the filtration—about 7 or 8 percent in each case. The spectral absorption of the extracts from the corn plant before filtration indicated the presence of more pigmented impurity in them than in the extracts from freshly cut green alfalfa or bluegrass and more pigment was removed from these corn extracts as a result of the adsorption filtration. These results will be presented in a later paper in connection with a study of the relation of the pigments in corn silage to those in the plant material from which it was made.

From the data in table 10, it is evident that the separation of the carotene from the pigmented impurity by adsorption filtration was accomplished with these extracts from hays and corn silage with very little or no loss or alteration of the pigments present, although changes in absorption of the magnitude of those reported by Gillam and El Ridi (*6*) with this column would have been negligible in this connection and would not have been detected with the equipment that was used.

Although the results in table 9 indicate that 11 to 32 percent of the absorption of the unfiltered carotene extracts at wave length 450 mμ was due to colored impurities in them, these results do not furnish definite evidence as to the nature or source of this impurity. Its properties resemble those of complex carotene oxidation products, or possibly, especially with corn silage, a mixture of such substances with decomposition products similar to those reported, in the work referred to on page 638, to be formed by the action of acids on xanthophyll (*18*, *23*.)

Regardless of the nature of these impurities, the question naturally arises as to whether they occur in the hays and silages and whether a correction for them should be deducted from the writers' regular carotene figures, or whether this pigmented material has been formed in the process of analysis, possibly from carotene, and that therefore the writers' carotene figures are too low. This question is still not finally settled, but the evidence at hand favors the view that these colored impurities occur in the hays and silages, that they are not entirely absent even from freshly cut plant materials, and that they are not formed to a considerable extent in the process of analysis.

The pigmented impurity removed by adsorption filtration, as described previously, from the carotene extracts from the hays is readily altered when a more highly activated adsorbent than that made from magnesium oxide is used; but it would seem from the data in table 9 that under the above-mentioned conditions this impurity has very much the same spectral absorptive properties regardless of the kind or quality of the hay from which it is obtained. It is possible, therefore, to calculate roughly, using average absorp-

tion data, the proportion of the absorption at a given wave length (e. g., wave length 450 mμ) that is due, on an average, to the presence of this colored impurity in the original unfiltered carotene extracts from the different types of hay. The results of such calculations are shown in table 11. A similar calculation with the unfiltered carotene extracts prepared from corn silage, according to methods A or B, indicates that on an average the proportion of the absorption at wave length 450 mμ that is due to these colored impurities is 30 percent or more.

TABLE 11.—*Average percentage of the absorption at wave length 450 mμ that is due to pigmented impurities in carotene extracts prepared from hays and corn silage according to methods A or B*

Kind of feed and grade [1]	Lots of feed included in average	Average calculated impurity in carotene extracts
	Number	*Percent*
Alfalfa hay:		
U. S. No. 1	12	18
U. S. No. 3	4	38
Timothy hay:		
U. S. No. 1	6	24
U. S. No. 3	12	40
Corn silage	12	30

[1] Grades determined by representatives of the Bureau of Agricultural Economics.

One might, from calculations similar to the above, correct in a rough way by making the appropriate deduction from the results for the carotene content of hays and corn silage as determined by methods A and B. The data given in tables 15 to 21 are not thus corrected, for in their present form they seem to be comparable to similar data from other laboratories. They express roughly the relative amounts of carotene in these feeds and thus constitute a very useful and practical guide in feeding (p. 660).

COMPARISON OF DIFFERENT METHODS OF DETERMINING THE CAROTENE IN CATTLE FEEDS

A number of methods for determining the carotene content of cattle feeds have been used in different laboratories. Some of these methods have been compared in the Beltsville laboratory, and the results are presented here together with other work which indicates somewhat the extent to which results of this sort may be comparable.

METHODS OF ANALYZING FRESHLY CUT PLANT MATERIALS FOR CAROTENE

The writers have compared method B with other procedures for determining the carotene content of freshly cut plant materials, as follows:

Procedure No. 1.—Freshly cut alfalfa was immediately frozen with solid carbon dioxide in the field, then dried in a vacuum pan cooled with carbon dioxide, and ground and analyzed as in method A. This procedure gave 356 μg of carotene per gram of dry material, as compared with 362 μg for samples done by method B, in which alcohol that was not initially cooled was used. The difference is not significant.

Procedure No. 2.—Fresh green material was put in a vacuum chamber at 100° C. within 5 minutes of cutting, dried in a stream of air at this temperature and at 7-cm pressure, and then ground and treated as in method A. Three lots of alfalfa were analyzed in this way. This procedure gave an average carotene content of 323 μg per gram of dry matter, as compared with 343 μg for samples done by the same method B procedure as was used in procedure No. 1. When other samples were dried in a stream of air at 60° instead of 100° C., much lower results were obtained (72 percent lower in one case and 90 percent in another).

Procedure No. 3.—Peterson, Bohstedt, Bird, and Beeson reported (*34*) that they were able to extract more carotene from freshly cut alfalfa if they acidified the alcohol they used in extracting it. They furnished the writers a description of the method they used, which involved refluxing the freshly cut plant material with alcohol containing 0.5 ml of concentrated hydrochloric acid per 100 ml of alcohol. In table 12 are shown the quantities of carotene found in the alfalfa plant by the method used by Peterson, et al., by method B in which the material was initially frozen at −60° to −70° C., and by this latter procedure with 0.5 ml of concentrated hydrochloric acid added per 100 ml of the alcohol that was used in this freezing mixture. The results show that a very much lower figure was obtained for the carotene when acid was used in its extractions than with the usual procedure employed by the writers.

TABLE 12.—*Effect of the presence of mineral acids on the quantity of carotene found in freshly cut alfalfa*

Lot, or sample No., of alfalfa analyzed	Date of cutting	Carotene per kilogram found when sample was analyzed by—		
		Method B, using alcohol at −60° to −70° C.	Method B with 0.5 ml concentrated HCl added per 100 ml of ethanol, using alcohol at −60° to −70° C.	Method used by Peterson, Bohstedt, Bird, and Beeson (*34*)
		Milligrams	*Milligrams*	*Milligrams*
1	July 18, 1935	166	129	42
2	July 30, 1935	280	54	113

Very great difficulty was encountered in the ligroin-methanol separation with the acid-treated samples for which the results are given in table 12. The spectral absorption of these extracts was, therefore, determined at a number of wave lengths. This was also done with the pigments that were removed from the carotene fraction by the methanol in this separation after this carotene fraction had been washed sufficiently to remove the xanthophyll completely. These data are shown in table 13.

TABLE 13.—*Effect of the presence of mineral acids in the extraction of carotene from alfalfa; the spectral absorption of the carotene fraction and of the pigments extracted from it by 92-percent methanol after removal of the xanthophyll*

Alfalfa hay sample No.	Fraction or pigment tested	Procedure used in preparing fractions or pigments	Relative spectral absorption at wave lengths of—[1]					
			440 mμ	445 mμ	450 mμ	460 mμ	470 mμ	480 mμ
			Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1 and 2	Carotene fraction	Method B, no HCl	82.4	98.1	100.0	92.8	84.9	87.1
		Method B, plus HCl	107.3	100.0	100.0	85.2	75.3	76.0
2	Methanol extract [2]	do		102.0	100.0	83.3	79.6	77.8
1	Carotene fraction	Peterson, et al. (*34*)	101.5	99.0	100.0	82.2	75.1	71.1
2	do	do		100.0	100.0	98.4	83.4	85.5
1 and 2	Methanol extract [2]	do	89.3	102.3	100.0	79.8	79.3	73.4

[1] Expressed in percentage of the absorption at wave length 450 mμ.
[2] These methanol fractions were obtained by repeatedly washing the carotene fractions after the latter had been washed a sufficient number of times to completely remove the xanthophyll.

The results in table 13 leave no doubt that the material that was removed from the carotene in the washing of the acid-treated samples was not carotene; but indicate, instead, that considerable quantities of this same material still remained in the carotene fraction. On the other hand, the spectral absorption of the carotene fraction with the samples that were done by the regular procedure of the Beltsville laboratory, was, as pointed out previously, in very close accord with that for beta-carotene. Apparently, in this laboratory the use of acid leads to a destruction of carotene. This is in accord with the early observations on the effect of acids on carotene and xanthophyll (*44*); and the more recent work of Kuhn and coworkers (*18*, *23*) would lead one to expect in addition a similar—in fact a greater—alteration of the xanthophyll, and difficulty similar to that encountered, in removing these products from the carotene.

METHODS OF DETERMINING CAROTENE IN HAYS

Methods A and B have been compared with the following procedures for the determination of the carotene in hays:

Procedure No. 1.—Samples were done by method A, omitting the initial soaking in alcohol. Three lots of alfalfa hay analyzed in this way averaged 19.5 mg of carotene per kilogram (air dry), and by method A, 21 mg.

Procedure No. 2.—Samples were done by method A, omitting the initial soaking in alcohol, but using 1 percent of ethanol in the low-boiling ligroin used in extraction. Four lots of hay analyzed by this method averaged 18.7 mg of carotene per kilogram (air dry) and by method A, 18.5 mg.

Procedure No. 3.—The ground hay sample was extracted six times as in method A except that acetone was used in place of the ethanol and ligroin; and the carotene remaining in the hay residues was finally washed out with ether as described by Schertz (*38*). The acetone was washed out of this mixture by treatment with water (*38*), the ether removed in vacuo, and the pigments taken up in low-boiling ligroin, saponified, etc., as in method A. Three lots of hay analyzed in this way averaged 20.0 mg of carotene per kilogram (air dry), and by method A, 21 mg.

Procedure No. 4.—Hay that had been soaked in alcohol as in method A, was extracted with low-boiling ligroin in a Soxhlet extractor. Four lots of hay analyzed in this way averaged 11.4 mg of carotene per kilogram (air dry) and by method A, 12.3 mg.

Procedure No. 5.—Hay was analyzed by method B, except that the chlorophyll in the ligroin solution was saponified with an equal volume of 5-percent KOH-ethanol at 40° C. for 3 hours according to the procedure of Kuhn and Brockmann (*18*) for the saponification of xanthophyll esters. Six carotene extracts from alfalfa hays that were done in this way gave 3.45 μg of carotene per milliliter; and method B with four regular saponifications, gave 3.50 μg. With three samples of alfalfa hay that were saponified by the procedure of Kuhn and Brockman and by method A with four saponification treatments, the average figures were 3.94 μg and 3.72 μg per milliliter of extract, respectively. These results would indicate (1) that little or no xanthophyll ester occurs in the carotene extracts from alfalfa hays when analyzed by the methods used here; and (2) that the shaking and aeration in saponifying, as done in routine work here, is probably not harmful.

Procedure No. 6.—Ninety-percent was compared with 92-percent methanol in the ligroin-methanol separation. Samples that were washed eight times gave 31.1 mg and 30.6 mg per kilogram of hay, respectively. Other samples from this same hay (alfalfa) that were washed 15 times gave 30.3 mg and 27.7 mg, respectively. It is clear that the last figure is likely to represent most nearly the actual carotene content of this hay, as pigmented material was clearly visible in most of the additional 92-percent methanol washings, the amount of it far exceeding that under similar conditions with pure carotene and decreasing with successive washings as, of course, would not occur perceptibly with carotene itself.

Procedure No. 7.—Guilbert's method (*7*). In this procedure 95-percent ethanol (by volume) was used in preparing the alcoholic-potassium hydroxide solution. This alcohol was treated with silver nitrate and potassium hydroxide to remove aldehydes, although the error due to resins from this source appears to be negligible. The ether extract was not brought to "dryness," but was concentrated in vacuo to remove the ether. Besides washing finally with 90-percent methanol, as recommended by Guilbert, additional samples were done by using 92-percent methanol as is usual here. Controls were run with alcoholic potassium hydroxide alone to determine the spectral absorption of the resins from this source. The extracted hay residues were re-extracted and this extract analyzed in the same way as the original extracts. These controls and extracts from re-extraction were read at wave lengths 430, 450, and 480 mμ. Several lots of hay were analyzed; the results in table 14 for one lot of hay, are typical.

TABLE 14.—*Comparison of results by Guilbert's method and by method B for the determination of carotene in hay*

[The extinction (E) is calculated to 1-cm layer for a 5-g sample and 50-ml volume]

Extracts, controls, and residues	Extinction coefficient and relative spectral absorption at wave lengths of—					
	430 mμ		450 mμ		480 mμ	
	E	Relative absorption [1]	E	Relative absorption [1]	E	Relative absorption [1]
Extracts washed 15 times:						
With 92-percent methanol:		*Percent*		*Percent*		*Percent*
Method B	0.524	79.8	0.657	100	0.536	81.6
Guilbert's method [2]	.540	79.1	.683	100	.550	80.5
With 90-percent methanol:						
Method B	.590	79.1	.746	100	.616	82.6
Guilbert's method [2]	.598	80.2	.746	100	.629	84.3
Resin in ligroin phase from KOH-ethanol control [3]	.065	130.0	.050	100	.024	48.0
Second extraction of hay residues: [4]						
Washed with 92-percent methanol	.026	81.2	.032	100	.027	84.4
Washed with 90-percent methanol	.048	85.7	.056	100	.042	75.0

[1] Expressed in percentage of the absorption at wave length 450 mμ.
[2] There is apparently no evidence of resinous material from the heating of the KOH and ethanol in these samples.
[3] Done by Guilbert's method, washing 15 times with the 90-percent methanol.
[4] Done by Guilbert's method in both cases.

It is obvious that this method and method B give very similar results with alfalfa hay when the extracts are similarly and adequately washed with methanol; the carotene is about equally completely extracted from the hay by these methods; and the discrepancies in the spectral-absorption data with these extracts as compared with those of beta-carotene, are practically the same by both methods. The data are from a hay that had lost about 50 percent of its carotene in storage since it was purchased, and the extracts with both methods obviously contain considerable amounts of pigments other than carotene. If these pigmented impurities had not been present in this hay, it is obvious from the data in table 14, showing the effect of using 90- and 92-percent methanol in the separation of carotene and xanthophyll, that this variation in the method of washing would have produced no difference in the results obtained.

CAROTENE CONTENT OF MARKET HAYS

ALFALFA HAYS

Table 15 shows the United States grade, color, and carotene content of 46 lots, or purchases, of alfalfa hay analyzed in the Beltsville laboratory. These hays were field-cured and baled. At the time of purchase they were inspected by representatives of the Division of Hay, Feed, and Seed of the Bureau of Agricultural Economics. The color, leafiness, time of cutting, and any other observations that were made regarding the condition of the hays, are noted in the table. The color and leafiness of some of the lots of hay was determined by inspection of the lot as a whole, but for other lots these factors were determined separately in the bales that were analyzed for carotene. The inspector examined some of these bales at Beltsville, but very

often they were sent to the laboratory of the Division of Hay, Feed, and Seed for more accurate examination.

TABLE 15.—*Carotene content of the various market grades of alfalfa hay*

PURCHASED AS U. S. NO. 1

Laboratory designation of each lot, or purchase, of hay	Natural green color	Leafiness	Stage of maturity at cutting	Carotene content per kilogram (air-dry basis)
	Percent	*Percent*		*Milligrams*
50	60–65	43	One-half in bloom	37
Test B [1]	85			121
Calf B [1]	75			37
57	72	56	One-fourth to one-half in bloom	31
58	73	51	Early bloom	35
59	65	50	do	31
64 [2]	60	40		43
H–O–1	70			41
H–O–1a	70			82
70				44
74 [3]	66	42	One-tenth in bloom	22
76	71	44	One-tenth to one-half in bloom	48
79	65	45	Full bloom	42
Mat. B., Oct. 17, 1936	85	45	do	63
Shop, medium, Nov. 5, 1936	67	40		35
Shop, low, Oct. 19, 1936 [4]	62	40		19
81	70	40–42	One-half in bloom	27
84	80	45	Bud	47
85	75	40	do	49
86	65–70	40–45	One-tenth to one-half in bloom	32
89	65–70	40	Full bloom	32
90				38
93				20
75: [5]				
Sublot A	75	45	One-fourth in bloom	57
Sublot B	65	43	do	8
Average	70	44	do	32
77: [6]				
Sublot A	70	45	One-half in bloom	48
Sublot B	60	30	Full bloom	17
Average	67	40		38
80: [7]				
Sublot A	70	48	One-fourth in bloom	54
Sublot B	67	42	One-half in bloom	72
Average	68	45		64
Average of 26 hays as purchased				42.7
Average of 27 hays that graded U. S. No. 1 in color only [8]				42.8

[1] Not graded officially; but classed as "very green."

[2] The bales in lot 64 contained some hay that was brown and that was graded U. S. No. 3. The No. 3 hay made up about 29 percent of the lot and contained 1.8 mg of carotene per kilogram. The remainder of the hay, an average of 70 percent per bale, was graded U. S. No. 1 and contained 60.6 mg of carotene per kilogram. The carotene content shown in the table is for a composite sample from 10 bales.

[3] Some of the bales in lot 74 were "set" or "packed" and looked as if they had been baled too soon. There were also yellow leaves that might have been the result of leafhopper infestation. The odor seemed normal.

[4] This sample had been stored from April to October in a rather dark barn loft.

[5] Lot 75 as purchased consisted of about equal portions of sublots A and B. The hay in sublot B had a sweated or ground odor, indicating that some decomposition or fermentation had occurred. There was no evidence of this condition in sublot A.

[6] Lot 77 as purchased consisted of two-thirds of sublot A and one-third of sublot B. There was no evidence of fermentation in sublot B.

[7] Lot 80 consisted of sublots A and B in the ratio of 15:19. The stalks in sublot A appeared to be finer and smaller than those in sublot B.

[8] Includes lot 73.

TABLE 15.—*Carotene content of the various market grades of alfalfa hay*—Continued

PURCHASED AS U. S. NO. 2

Laboratory designation of each lot, or purchase, of hay	Natural green color	Leafiness	Stage of maturity at cutting	Carotene content per kilogram (air-dry basis)
	Percent	*Percent*		*Milligrams*
60 [9]	45	45		19
H–O–2 [10]	55	56		14
H–O–2a	58	50		13
51	50–60	50		15
54	51	43		12
Mat. B. Nov. 12, 1936	45	40		20
73: [11]				
Sublot A	38	42		15
Sublot B	67	30		46
Sublot C	81	30		77
Average	63	34		46
Average of 7 hays as purchased				19.9
Average of 7 hays that graded U. S. No. 2 in color only.[12]				14.9

PURCHASED AS U. S. NO. 3

71.5	38	20		11
56	1	41		1
48	10–25	45	Bud to early bloom	11
51 [13]	15	25	In bloom	8
63 [13]	20	30	do	3
66	15–35	35–38		5
67	15–25	30–35		4
71 [13]	5–10	20–25	One-tenth in bloom	1
78	0– 6	35–48	Bud	2
87	15	15	Early cut	3
88	20	15		1
91				4
92				4
Average of 13 hays as purchased				4.5
Average of 12 hays that graded U. S. No. 3 in color only.[14]				4.0

[9] Lot 60 as purchased was graded U. S. No. 1 (color 60 percent and leafiness 50 percent) but upon reinspection, the bales that were analyzed were graded as shown and they appeared to be representative of the lot. 1 bale that appeared "set" but not caked, indicating that fermentation had occurred in it, was graded 40 percent in color and contained 10 mg of carotene per kilogram. 2 other bales were caked, but in sampling them 1 bale had been drilled from an end that was much greener and less affected, if at all, by fermentation. These 2 bales contained 24 mg of carotene per kilogram.

[10] This was a single bale of hay. On Feb. 12, 1935, it was graded 63 percent in color and the carotene content was 14 mg per kilogram; on Apr. 5, 1935, it was graded 48 percent in color and the carotene content was 15 mg per kilogram. The datum in the table for color is the average of these determinations.

[11] Lot 73 was not uniform. The bales in sublot A looked as if the hay had heated in the bale as a result of bailing too soon after cutting. There were about equal amounts of each sublot.

[12] Omits lot 73 and includes lot 71.5.

[13] These hays were badly weathered.

[14] Omits lot 71.5.

The method of color measurement used in the Bureau of Agricultural Economics is described by Nickerson (*33*), who gives the Munsell hues associated with the various percentages of "natural green color," as expressed in hay inspection. According to the hay standards, described by the Bureau of Agricultural Economics (*43*), a sample of alfalfa hay may be sufficiently high in percentage of natural green color to be in grade 1, but may be placed in grade 2 or 3 because its leaf content is low (table 15, lot 73, sublots B and C; and lot 77, sublot B); or it may contain sufficient leaves to be in grade 1, but be placed in grade 2 or 3 because its percentage of natural green color is low (table 15, lots 60; H–O–2; H–O–2a; 73, sublot A; 51; 54; 56; and

48). Also a lot, or purchase, of hay is frequently not uniform, some bales being quite different from others (table 15, lots 75, 77, 80, and 73). The data in table 15 are according to the grading of the lot, or purchase, of hay as a whole; but, where the bales in the lot differed in this way, the lot, or purchase, of hay was separated into uniform sublots and each sublot was analyzed. The data for these sublots are also given in the table.

Attention should be called to lots 74; 75, sublot B; 60; and 73, sublot A. Fermentation or spoilage was evidenced in all of these hays by a caked or packed condition in the bale and by a tobacco, ground, or sweated odor.

In considering the data in table 15 one must bear in mind that besides the errors in the carotene determinations discussed above, there are relatively large and unavoidable errors in the determination of the leafiness and color of these hays. Despite these facts, an inspection of the data for the alfalfa hays in table 15, seems clearly to justify the following summary:

(1) There is a very decided difference between the average vitamin A potency of the No. 1, No. 2, and No. 3 grades of alfalfa hay as indicated by the carotene content, the average carotene content of the hays shown in table 15 being 42.7, 19.9, and 4.5 mg per kilogram, respectively, for these different grades. Since alfalfa hay is graded commercially mainly on the basis of its leafiness and color, the question arises as to whether these differences in carotene content are associated with either of these factors used in grading.

(2) The results in table 15 indicate that, although the carotene occurs largely in the leaves of the alfalfa plant (*36*) and is high in alfalfa leaf meals (table 16), the percentage of leafiness in field-cured, market hays plays a minor role in determining their carotene content. It is true that the average leafiness of the hays that are poorest in carotene is lower than the average leafiness of the others. Thus the average leafiness of the hays containing 1 to 11 mg of carotene per kilogram, as is the case with the grade 3 hays, is only about 30 percent, whereas the leafiness of the hays containing more than this amount of carotene is about 42 percent. Nevertheless, the average leafiness of the hays containing 12 to 20 mg of carotene per kilogram, as is the case with the grade 2 hays, is fully as great as that of the hays containing between 27 and 77 mg per kilogram; and there are hays that are low in their leaf content but high in carotene, and vice versa. Undoubtedly a large proportion of the carotene in these alfalfa hays occurred in the leaves, but the leaves, nevertheless, may or may not be rich in this pigment.

A few samples of machine-dried alfalfa leaf meals have been analyzed for carotene and the results are shown in table 16.

TABLE 16.—*The carotene content of machine-dried alfalfa leaf meals*

Sample No.	Greenness	Carotene content per kilogram (air-dry basis)	Sample No.	Greenness	Carotene content per kilogram (air-dry basis)	Sample No.	Greenness	Carotene content per kilogram (air-dry basis)
	Percent	*Milligrams*		*Percent*	*Milligrams*		*Percent*	*Milligrams*
1	97	155	4	74	76	7	80	212
2	64	122	5	85	244			
3	51	90	6	85	160	Average		151

The high carotene figures for these meals are in accord with results from other laboratories. The spectral absorption data in table 8 are of interest in this connection, being in good agreement with those shown for beta-carotene (second international standard, table 8).

(3) From data in table 15, it is obvious that any evidence of spoilage such as a caked or set condition in a bale of hay or a tobacco, sweated, or ground odor (table 15, lots 60; 73, sublot A; 74; 75, sublot B) may be taken as an indication of a decidedly low carotene content. This condition may be associated with a low percentage of green color (lots 60; and 73, sublot A); but apparently it also occurred in hays that were not graded particularly low in this respect (lots 74; and 75, sublot B).

This fermentation in hays is the result of baling them before they are sufficiently dry; but overexposure to sunlight in drying has also been found to be destructive of carotene and must be avoided if hays of high carotene content are to be obtained. The optimum conditions at which hays should be placed in storage have been studied by Shepherd and Woodward (*39*).

(4) The percentage of natural green color in alfalfa hays is by far the most important, general, and reliable index of the carotene content. This is obvious from the results shown in figure 1, as well as from those in table 15.

If one disregards all lack of uniformity and all evidence of fermentation in the lots of alfalfa hay shown in table 15 and grades these hays on the basis of color alone, the average carotene content of the hays placed in grade 1, 2, or 3 is 42.8, 14.9, and 4.0 mg per kilogram, respectively. The hays in grade 1 would on this basis contain 19 to 121 mg of carotene per kilogram of hay; those in grade 2, 11 to 20 mg; and those in grade 3, 1 to 11 mg.

On the other hand, if each uniform lot or sublot of hay shown in table 15 is graded according to its color, and those hays in which spoilage occurred are omitted, then the average carotene content of the hays placed in grade 1, 2, or 3 is 45.7, 14.2, and 4.1 mg per kilogram, respectively. The carotene content, on this basis, would vary from 17 to 121 mg per kilogram, 11 to 20 mg per kilogram, and 1 to 11 mg per kilogram, respectively, for grades 1, 2, and 3. These results are shown graphically in figure 1.

The two hays of lowest carotene content that on the latter basis would be in grade 1 are of interest. They are lot 77, sublot B and a single bale of hay designated as "Shop, low, Oct. 19, 1936." The former was graded barely 60 percent in green color and contained only 30 percent of leaves; the latter was from a lot of hay (lot 80, sublot A) that was purchased and first analyzed during April 1936. From April to October this hay had been stored in a rather dark barn loft. Under these conditions of storage the percentage loss of carotene is much greater than that of green color (*46*); and the bale that was analyzed in October is apparently no exception. It was 65 percent lower in carotene content, but only about 10 percent lower in green color than those bales that were analyzed in April. This result is the only one included in table 15 for a hay that was stored in this way after being purchased. It illustrates a general condition which tends materially to alter the relation between the color and the carotene content of alfalfa hays. Hay that has been stored under these

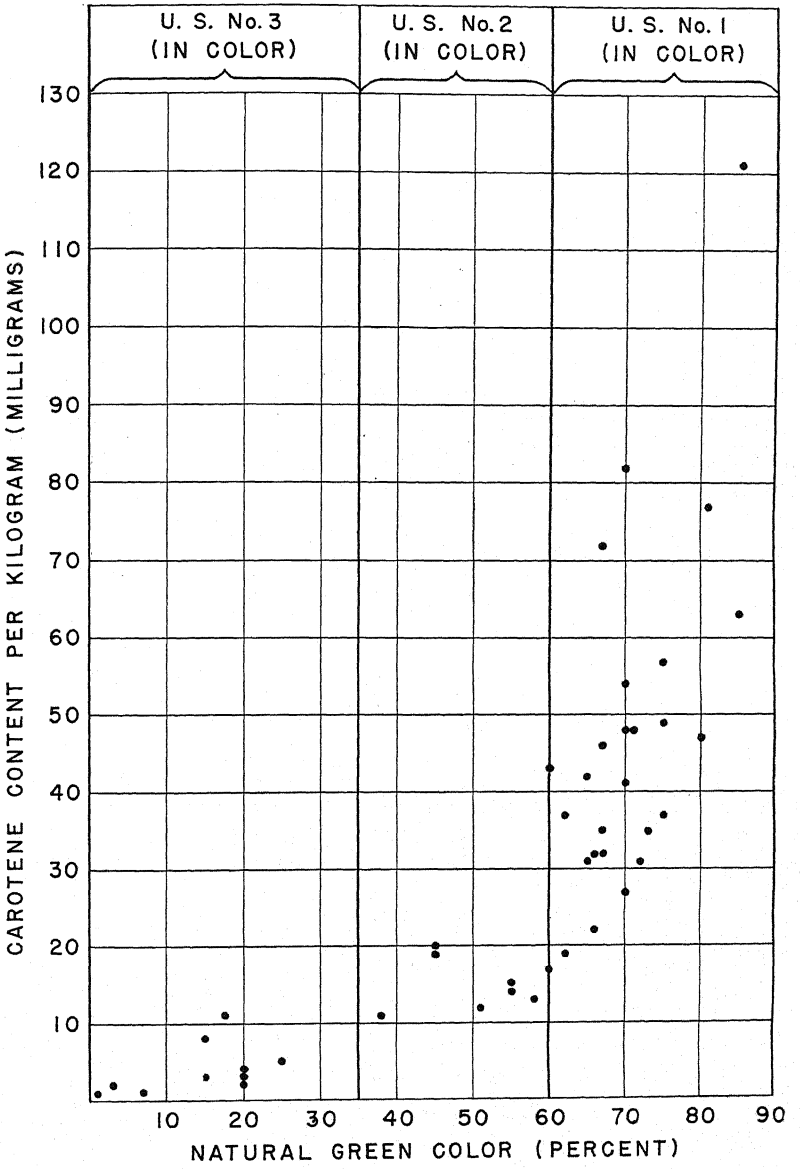


FIGURE 1.—Relation between the United States grade (in color only) and the carotene content of the alfalfa hays analyzed at the Beltsville laboratory. No hays in which spoilage had occurred are shown, and only uniform lots or sublots of hay are included.

conditions may have a much lower carotene content, as compared with its color, than it had soon after it was cut. Obviously, there is a very useful practical relation between the color and carotene content of alfalfa hays; but, of course, no definite quantitative relation between these factors can be expected.

It is interesting to note that all the 27 uniform lots or sublots of alfalfa hay with a "natural green" color of 60 percent or more (fig. 1) contain 20 mg or more of carotene per kilogram; and that a hay with this minimum concentration of carotene, when fed at about 50 percent of the ration, furnishes close to the minimum amount of vitamin A that a cow requires for normal reproduction (*35*) (p. 660). It is interesting to note further that before either of these facts was known, hay-grading authorities had selected this percentage of natural green color as the minimum for U. S. No. 1 alfalfa hay.

TIMOTHY HAYS

Table 17 gives the United States grade, color, and carotene content of 40 lots or purchases of timothy hay that have been analyzed in the Beltsville laboratory. Although the carotene content of the hays overlaps to some extent in the various grades, there is a distinct tendency for it to vary with the grade of the hay. The average carotene content of the timothy hays in grades 1, 2, and 3 is 20.6, 9.3, and 5.5 mg per kilogram, respectively. As the grading of timothy hays is based on the percentage of green color in them this means that, as with the alfalfa hays, the carotene content varies in general with the color. But the amount of this pigment varies from 8 to 36 mg per kilogram in the grade 1 hays; from 8 to 11 mg per kilogram in the grade 2 hays; and from 1 to 12 mg per kilogram in grade 3 hays. As would be expected, the timothy hays that were cut when ripe or overripe are uniformly low in their carotene content; whereas those hays that were cut in the prebloom stage generally contain more of this pigment.

TABLE 17.—*Carotene content of various market grades of timonthy hay*

PURCHASED AS U. S. NO. 1

Laboratory designation of each lot	Natural green color	Stage of maturity at time of cutting	Carotene content per kilogram (air-dry basis)
	Percent		*Milligrams*
40	50		20
46	48–50	Prebloom	23
47	45	do	22
51	47	do	[1] 8
57	55	do	19
61	62	Bloom and early bloom	16
73	85	Late bloom	36
75	55–60	Before bloom	21
80	60–65		19
43:			
7 bales	50		21
1 bale	50		30
Average	50		22
Average of 10 lots			20.6

[1] Average of 8 agreeing determinations on 5 bales of hay. The bales were carefully graded and showed no evidence of spoilage. Meigs and Converse report (unpublished) that they found this hay inadequate in its carotene content to prevent the abnormal calving by a cow that received it as approximately 50 per-

TABLE 17.—*Carotene content of various market grades of timothy hay*—Continued

PURCHASED AS U. S. NO. 2

Laboratory designation of each lot	Natural green color	Stage of maturity at time of cutting	Carotene content per kilogram (air-dry basis)
	Percent		*Milligrams*
39	35–40	Early dough	8
44	35–40	Quite immature	9
48	35	Early dough	11
Average			9.3

PURCHASED AS U. S. NO. 3

37	20–25	Ripe	1
42	15–20	Late dough	10
38	10		1
45	20	Ripe	4
53			2
56	15–20	Overripe	6
64	22	Ripe	8
65	8	do	7
66			5
60	15–20	Overripe	4
69	20–25	do	5
70	15–20	do	3
72	15–30	do	11
74	20	Ripe	12
76	10	Ripe (headed for seed)	4
77	10	Late dough, weathered	3
78	20	Late dough	5
79	10	do	5
81	20–25	do	2
82	15	Ripe	3
83	15	Late dough	4
84	20	Ripe	10
85	15	do	9
86	15	do	11
87	15–20	do	6
88	5	Ripe and weathered	3
89			5
Average			5.5

CLOVER HAYS

Only four samples of clover hay have been analyzed for carotene in the Beltsville laboratory. They were all graded at the time of purchase as either U. S. No. 1 Clover or U. S. No. 1 Clover Light Timothy Mixed. The carotene content of the four samples was 11, 14, 24, and 43 mg per kilogram of hay, respectively.

PRACTICAL SIGNIFICANCE OF CAROTENE DETERMINATIONS IN CATTLE FEEDING

Most of the hays for which the carotene content is given in this paper have been fed to cattle at the Beltsville station, largely in experiments in which they were practically the only source of vitamin A in the ration. Converse, Wiseman, and Meigs (*3*) have studied the effect of these hays upon the nutritive value of the milk of cows, while Meigs and Converse (*29*) have investigated the value of the hays as a source of the vitamin A required by dairy cows for various functions, particularly for growth and reproduction. Their results indicate that there is practically a definite relation between the

carotene content of hays (as determined by routine analytical methods) and the value of these hays as a source of vitamin A.

They found that the reproductive function in the cow is sensitive to a deficiency of vitamin A in the ration, and that the amount of vitamin A required for this function quite likely determines the minimum amount of vitamin A or its precursors that it is advisable to feed to a cow. On the other hand, it is clear from their work that there is a corresponding range of carotene content, as determined in the hays that they used, that meets these requirements. They report failure in reproduction with all of their cows, when U. S. No. 3 alfalfa or timothy hay constituted approximately 50 percent of the ration and was very nearly the only source of vitamin A in the ration for a period of at least 5 months before the cows calved. The calves were born dead, blind, or so weak that they died soon after birth. When U. S. No. 1 alfalfa hay was similarly fed, there was normal success in reproduction; and with hays that were intermediate in carotene content—possibly ranging from 10 to 20 mg or 15 to 20 mg of carotene per kilogram of hay—the results were variable.

In a subsequent report based on these experiments of Meigs and Converse, which are still in progress, Rogers (*35*) states:

> If the carotene content of the hay of the ration, where fed at about one-half of the ration, is as high as 20 parts per million calving is normal; if it is as low as 14 the results are uncertain and at levels as low as 5 parts per million there are no normal calvings.

Apparently, with cows fed as here described, those hays listed in tables 15 and 17 that contain 20 mg or more of carotene per kilogram, as determined by methods A and B, would supply close to the minimum amount of vitamin A required for normal reproduction; and those containing 14 mg or less would not do so. The carotene figure, therefore, as determined for a hay by the ordinary routine procedures described in this paper, is a decidedly useful index of practical value in cattle feeding.

As shown in the earlier part of this paper, this statement is true whether the carotene is determined according to methods A and B, described in this paper, or according to comparable methods developed in other laboratories. There is, therefore, available a considerable amount of comparable information on the suitability, from this standpoint, of various materials for cattle feeding and on the vitamin A requirements of cattle. However, just as numerous factors affect the efficient utilization of the energy or of the protein of the ration, the same may very well be found to be true of the utilization of vitamin A and its precursors (*13*), when these results are applied in practice.

In a recent paper, Guilbert, Miller, and Hughes (*9*) present the results of a study of the amount of carotene and vitamin A required by cattle to prevent night blindness. They report that a minimum of 25 to 30 μg of carotene per kilogram of body weight, or 6 to 8 μg of vitamin A, is necessary in the ration for this purpose; but they found that pregnant, nonlactating cows, maintained on these minimum levels throughout gestation, uniformly gave birth to weak calves that died soon thereafter. Increasing the carotene intake to three or four times this minimum during the last month of gestation consistently resulted in normal calves at birth. These workers also report that, with cows maintained for long periods during lactation on approxi-

mately the minimum level of carotene that would prevent night blindness, the milk was practically devoid of vitamin A and nursing calves developed symptoms of vitamin A deficiency and died.

This work clearly demonstrates that night blindness, as detected in cattle by the method that these workers used, is not a criterion by which to judge practically the adequacy of a ration as a source of vitamin A for dairy cattle, but that the minimum quantity of this factor that it is advisable to feed these animals certainly is more nearly the amount Meigs and Converse found to be necessary for normal calving (p. 660).

CAROTENE RESULTS WITH MISCELLANEOUS FEEDS

A number of miscellaneous feeding materials have been analyzed by means of the carotene methods described on pages 638–639 and the results are included in table 18. It must be borne in mind that the figures for the grains, although they include the carotene present, are not a measure of their vitamin A value.

TABLE 18.—*Carotene found in various feeding materials analyzed in the Beltsville laboratory*

Feeding material	Samples analyzed	Carotene per kilogram	
		Range	Average
	Number	*Milligrams*	*Milligrams*
Wheat straw (air dry)	1	----------	(1)
Corn stover (air dry)	2	2– 6	4
Kentucky bluegrass (dry weight)	4	424– 662	567
Alfalfa, freshly cut (dry weight)	9	271– 412	[2] 334
Garden carrots, yellow: [3]			
Green weight	11	36– 132	91
Dry weight	11	268–1,692	914
Corn grain, yellow	6	3– 9	[4] 5
Wheat bran	2	0.12– 0.23	[4].18
Linseed meal	3	0.23– 0.29	[4].26
Soybean meal	1	----------	[4].23

[1] Less than 2 mg per kilogram.
[2] 1 sample of alfalfa, obtained from a plot that was infested by leafhopper, contained 166 mg per kilogram. The sample was selected as the best hay in the plot.
[3] Garden carrots purchased on market for feeding experiments.
[4] This figure includes the carotene along with other pigments, such as cryptoxanthine, which go more or less into the carotene fraction.

CAROTENE CONTENT OF CORN SILAGE

Since 1934, this laboratory has made about 30 determinations of the carotene content of the silage used in feeding the cattle in the herd employed in nutrition experiments at Beltsville. Each determination has consisted of the analysis of several aliquots, frequently from each of several large samplings. In some instances monthly composite samples have been made from samplings taken twice daily for the month, each daily sampling being kept in an ice box in a closed fruit jar until the monthly composite was made and analyzed. The results of these silage determinations are given in table 19.

TABLE 19.—*Carotene content of miscellaneous samples of corn silage*

Silage sample No.	Date of analysis	Carotene content per kilogram—		Silage sample No.	Date of analysis	Carotene content per kilogram—	
		On basis of silage as fed	On basis of dry weight			On basis of silage as fed	On basis of dry weight
		Milligrams	*Milligrams*			*Milligrams*	*Milligrams*
1	Mar. 8, 1934	12	48	20	Nov. 14, 1936	7	22
2	Mar. 14, 1934	12	48	21	Dec. 30, 1936	19	70
3	May 26, 1934	13	48	22	Feb. 10, 1937	18	60
4	July 9, 1934	3	13	23	Mar. 8, 1937	11	43
5	July 30, 1934	7	20	24	Mar. 15, 1937	9	28
6	Aug. 6, 1934	10	39	25	July 30, 1937	3	10
7	Sept. 4, 1934	16	60	26 [1]	March 1937	6	20
8	Sept. 17, 1934	11	39	27 [1]	April 1937	7	22
9	Nov. 17, 1934	1	4	28 [1]	May 1937	7	24
10	Jan. 5, 1935	37	146	29 [1]	June 1937	5	18
11	Mar. 4, 1935	40	156	30 [1]	July 1937	4	13
12	Mar. 9, 1935	28	111				
13	Mar. 16, 1935	29	103	Average		13.7	49.9
14	Mar. 21, 1935	36	122	Average, omitting samples 9 to 17, inclusive [2]		9.4	33.0
15	Mar. 25, 1935	10	35				
16	Sept. 5, 1935	25	98				
17	Sept. 20, 1935	8	29				
18	June 22, 1936	10	28				
19	June 25, 1936	7	20				

[1] Composite sample. 2 samples were taken from the silage as fed daily, kept in fruit jars in an ice box, and composited before analysis. The keeping of the samples in this way was tested out and was found not to lead to a destruction of the carotene. Later, however, evidence of spoilage was noted in some jars. On this account 4 of the 60 jars in April, 5 in May, and 1 in June, were discarded.

[2] Samples 9 to 17, inclusive, do not represent the results of average practice on the farm where this work was done.

The carotene content of the 30 samples of corn silage ranges from 1 to 40 mg per kilogram of the silage as fed, or from 4 to 156 mg per kilogram of dry weight. The average carotene content of these 30 samples is 13.7 mg per kilogram of the silage as fed, or practically 50 mg per kilogram of dry weight.

All the samples of silage listed in table 19, except Nos. 9 to 17, inclusive, represent the results of average farm practice on the dairy farm at Beltsville. The carotene in these samples ranges from 3 to 19 mg per kilogram of silage as fed, or from 10 to 70 mg per kilogram of dry weight; and the average carotene content is 9.4 mg per kilogram of silage as fed, or 33 mg per kilogram of dry weight. These samples are probably not sufficiently comparable in other respects to permit any conclusions regarding the relation between their carotene content and the duration of the period of storage in the silo. This matter is now under investigation.

The data for the samples of silage that represent average farm practice at Beltsville agree with those from other laboratories, and probably represent the results obtained frequently in practice. Lipman (*24*) reports an average of 15 International units of carotene per gram of silage for six samples that were analyzed at the New Jersey Agricultural Experiment Station. Krauss (*16*) reported 2.5 Sherman units of vitamin A potency per gram for one sample of silage; and in a subsequent paper (*17*) reported using silage containing 52 mg of carotene per kilogram of dry material.

It is obvious from the results obtained with corn silage that frequently the silage might be suspected of failing to supply enough carotene in the ration to furnish the vitamin A required for normal

reproduction. Meigs and Converse have in progress in the Beltsville laboratory an experiment to test this point. No results of their experiment have yet been published, but they have been kind enough to permit the following statements: Up to the present time there have been five calvings by cows on a ration of grain and corn silage. The silage was fed ad libitum and the grain was fed in amounts to supply adequate energy and protein. Of these five calvings, three have been normal. In the other two cases the calves died at 2 and 13 days of age respectively.[7] Where corn silage is to be used thus in the ration of a cow as practically the sole source of vitamin A, or is to be depended upon in large measure to furnish this factor in the diet, it is evidently important to know the conditions under which it may be produced with adequate carotene content.

Preliminary work of this sort was started in the fall of 1934, with the silage samples 9 to 17, inclusive (table 19), to learn something of the conditions that determine the carotene content of corn silage. A record was available of the varieties of corn that had been planted in that year. When the crop was cut in the fall the maturity and greenness of the corn were noted, a record was kept of the way it was handled, the weather conditions, etc., and from time to time as the chopped corn was put into the silo a large amount of the material was mixed in the silo and duplicate samples were taken. Aliquots from one of these duplicates were analyzed immediately for carotene and moisture. The other duplicate, amounting to 15 to 20 kg, was placed in a very porous burlap bag, left in the silo, and analyzed when it was uncovered during subsequent feeding. Nine bags were thus left in the silos. The results of this experiment are shown further in table 20.

TABLE 20.—*Relation between the carotene content of corn silage and the maturity and carotene content of the corn from which it was made*

Silage sample No.[1]	Date of analysis of silage	Condition of corn plant at cutting		Carotene content per kilogram (dry-weight basis)	
		State of maturity	Greenness[2]	Corn plant[3]	Silage
			Percent	*Milligrams*	*Milligrams*
10	Jan. 5, 1935	Ears 100 percent in milk, undented	100	115	146
11	Mar. 4, 1935	do	90	92	156
12	Mar. 9, 1935	do	90	94	111
Average		do		100	138
13	Mar. 16, 1935	Ears 50 percent in milk, 50 percent in dough	90	70	103
14	Mar. 21, 1935	do	90	90	122
16	Sept. 5, 1935	Some milk, but average ear slightly dented	75	74	98
Average				78	107
15	Mar. 25, 1935	Ears dented	40–50	21	35
17	Sept. 20, 1935	do	40	25	29
Average		do		23	32
9	Nov. 17, 1934	Ears dented, plant exposed to light frost[4]	20	6	4

[1] Samples 10 and 11 were from the same variety of corn; samples 12, 15, and 17 were from the same variety; and samples 9 and 13 were from the same variety.
[2] The portion of the plant that was estimated to be green.
[3] Carotene content at time the material was chopped for ensiling. The time and condition of exposure between the time the corn was cut in the field and the time it was chopped for ensiling varied for the different lots of corn.
[4] There was a light frost for 1 night, 8 days before this corn was cut.

[7] In addition to these calvings on this grain-corn silage ration there was one abortion that occurred much earlier than those that have been associated here with a deficiency of vitamin A in the ration.

In considering the data in table 20 it will be recognized that a number of factors which may have affected the carotene content of the corn from which these silage samples were made varied—e. g., the variety of the corn, its maturity and greenness, the weather conditions preceding and at the time of cutting, the period elapsing between the time the corn was cut and the time it was put into the silo, the weather conditions during this time, etc. The carotene content as found in the samples of silage may have been affected, in addition, by variations in the time that the silage was stored, the exposure of the bags near the top of the silo before they were removed, and numerous other conditions. It is impossible from the information in this preliminary experiment to accurately evaluate or rigidly eliminate many of these factors; but, despite this situation, it is clear that certain correlations in the data in table 20 are much more consistent and striking than others. Of these the following may be noted:

(1) The carotene content of the corn from which the lots of silage were made was, as might have been expected, most strikingly and consistently affected by the maturity of the corn and the proportion of the plant that was green at the time of cutting. In the three greenest and least mature samples of corn the carotene content varied from 92 to 115 mg per kilogram of dry weight, and averaged 100 mg; in three samples of corn that were slightly more mature the carotene content varied from 70 to 90 mg per kilogram and averaged 78 mg; whereas in the two samples of corn that were fully dented (but not frosted) and only 40 to 50 percent green the carotene content was 21 and 25 mg per kilogram of dry weight, respectively. The effect of a light frost upon the carotene content of the corn plant is well shown in sample No. 9.

(2) The carotene content of the silage samples shown in table 20 was, without doubt, definitely affected by that of the corn from which they were made. Corn having an average carotene content of 100 mg per kilogram of dry weight produced a silage with a carotene content of 138 mg per kilogram; corn having an average carotene content of 78 mg produced a silage with a carotene content of 107 mg; and corn having an average carotene content of 23 mg produced a silage with a carotene content of 32 mg; while the sample of frosted corn with a carotene content of 6 mg produced a silage that contained only 4 mg. Samples 15 and 17 appear to represent the results of average practice in the making of silage on the farm at Beltsville.

(3) It may be noted that the carotene content of the silage samples in table 20, with the exception of the frosted sample, is uniformly higher than that of the corn from which they were made. The corn with an average carotene content of 100 mg per kilogram produced silage with a carotene content apparently 38 percent higher; and for the two lots of corn with average carotene content of 78 and 23 mg, respectively, the apparent increase in carotene in the silage was 37 and 39 percent, respectively. Probably, the good agreement in these figures is largely a matter of chance; but it does indicate that the apparent percentage increase in carotene in the silage does not vary materially with the amount of carotene in the original plant material.

There were probably a number of changes in the pigments in the corn plant from the time it was cut in the field to the time samples of the freshly chopped material were taken from the silo, and also from

this time until the silage itself was analyzed. The increase in the amount of pigmented material in the carotene extracts from these silages, as compared with the corn from which they were made, may possibly best be regarded as the net result of at least more than one change going on in these pigments in the silo, and insofar as the writers are aware, there is no conclusive evidence indicating the nature of the material making up this net increase of pigment in the carotene fraction from the silage. It is true that Krauss (*16*), reported 2.5 Sherman units of vitamin A potency per gram in silage made from corn that contained only 1.25 Sherman units; but no details of this work were given.

Apparent increases in carotene content, similar to those just noted with ordinary corn silage, have been reported repeatedly in silage made by the A. I. V. method,[8] where it would seem much less likely that carotene might be produced by bacteria or in the continued metabolism of the cells of the ensiled plant; and, although it has been demonstrated that A. I. V. silages are rich in carotene and are potent sources of vitamin A, no one seems to have determined the nature or biological properties of the additional pigment that occurs in the carotene extracts from them. It is possible that this additional pigment may be carotene; but since, according to Kuhn and Brockmann (*18*), pigments that behave like carotene in analysis may be formed by the action of weak acids on xanthophyll, one must regard such an assumption with some reservation until further evidence is at hand. Also, since a similar acid reaction prevails during the storage of corn silage, this possible origin of the increase in pigment in corn silage over and above that in the corn from which it was made must be borne in mind.

The data discussed on pages 643–648, indicating that the carotene extracts obtained in the routine analysis of corn silage contain pigments other than carotene and that these so-called pigmented impurities account on an average for about 30 percent of the absorption at wave length 450 mμ, are of interest in connection with the data in table 20, which apparently show that more carotene exists in the silage than was in the corn from which it was made. It is of interest, further, to note that the average amount of pigmented impurity in these extracts is about equivalent to this apparent increase in the carotene content of the silage. Thus, regardless of the nature of the changes in the pigments during storage in the silo, apparently the full amount of carotene originally in the corn is well maintained.

In considering the data in table 20 from a practical standpoint, it may be pointed out that to cut corn when the ears are practically all in the milk stage entails a considerable loss of yield in dry matter per acre. Cutting corn at this early stage is probably unnecessary, however, for it will be noted that corn that was cut in the early dough stage, or when the kernels were slightly dented, still yielded a silage that was rich in carotene. In silage from corn that was cut when the kernels were fully dented and only 40 to 50 percent of the plant

[8] In the A. I. V. method, named for its sponsor A. I. Virtanen of Finland, certain acids are added to increase the acidity of the ensiled material.

was green, the carotene content was considerably reduced; and the practice of cutting corn at this stage is likely to lead to the production of considerable silage that is inadequate in its carotene content to supply the amount of vitamin A required by dairy cows for normal reproduction, especially when it is practically the sole source of carotene in the ration. The minimum amount of carotene, as ordinarily determined in corn silage, that it is necessary to feed a cow has never been definitely determined. Certainly, it would be well to feed enough silage to provide at least 100 to 150 mg of carotene per day during the dry period, and more would certainly be desirable.

SUMMARY

The methods for the determination of carotene that are now in general use in the laboratory of the Division of Nutrition and Physiology at Beltsville, Md., are described in this paper. According to these methods the carotene is separated from the chlorophyll and xanthophyll by a modification of the well-known Willstätter and Stoll procedure.

Data are presented here to show the errors that occur in the use of this procedure. The largest error in the determination of the carotene in hays and corn silage by this procedure was found to be due to pigmented impurities other than xanthophyll or chlorophyll in the final carotene extracts. The presence of such pigmented impurities has been demonstrated by a study of the spectral absorption and adsorptive properties of these extracts. The error from this source in determining the carotene in the extracts varied from about 11 to 32 percent in the case of the hays and corn silage. It was least with fresh green plant materials and with leaf meals and hays of high carotene content. The source of these pigmented impurities is still uncertain. Some evidence tends to indicate that they are not in general produced in the process of analysis, but this possibility has not been satisfactorily excluded.

Despite this error in the determination of the carotene in feeds and the uncertainty of the source of these pigmented impurities, the carotene content as determined by the usual routine methods now in use in the Beltsville laboratory is shown to be a very useful index of the vitamin A value of feeds of plant origin that are used in cattle feeding.

Data are presented on the carotene content of various kinds and qualities of market hays, the relation between their physical properties and carotene content, the carotene content of corn silage, some of the conditions affecting the carotene content of this silage, and the results obtained in applying these routine carotene-determination methods to fresh green plant materials, alfalfa leaf meals, various grains, etc. These results are summarized in table 21.

TABLE 21.—*Summary of results on the carotene content of cattle feeds, given in this paper*

Feed	Carotene content per kilogram			
	Dry weight		Weight as fed	
	Range	Average	Range	Average
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*
Green growing material:				
Bluegrass	424–662	567		
Alfalfa [1]	271–412	332		
Artificially dried material: Alfalfa leaf meals			76–244	151
Silage: Corn	4–156	50	1– 40	14
Alfalfa hay:				
Grade U. S. No. 1 in color			19–121	43
Grade U. S. No. 2 in color			12– 20	15
Grade U. S. No. 3 in color			1– 11	4
Timothy hay:				
Grade U. S. No. 1 in color			[2] 8– 36	21
Grade U. S. No. 2 in color			8– 11	9
Grade U. S. No. 3 in color			1– 12	5
Carrots (yellow garden)	268–1, 692	914	36–132	91
Corn stover (dry)			2– 6	4
Clover hay (U. S. No. 1)			11– 43	23

[1] Samples not infested by leafhopper.
[2] 1 apparently exceptional sample contained 8 mg of carotene per kilogram.

LITERATURE CITED

(1) BRICE, BROOKS A.
1937. A COMPENSATING CIRCUIT FOR BLOCKING-LAYER PHOTOELECTRIC CELLS. Rev. Sci. Instruments 8: 279–285, illus.

(2) CONVERSE, H. T., and MEIGS, EDWARD B.
1936. VITAMIN A REPLACES WHOLE MILK IN THE CALF RATION. (Abstract) Jour. Dairy Sci. 19: 435–436.

(3) ——— WISEMAN, H. G., and MEIGS, E. B.
1935. THE RELATION BETWEEN THE COLOR AND VITAMIN A OF BUTTER AND THE FEED OF THE COW. Amer. Soc. Anim. Prod. Proc. (1934) 27: 50–54.

(4) EULER, H. v., KARRER, P., and WALKER, O.
1932. ÜBER EIN OXYD DES CAROTINS. Helvetica Chim. Acta 15: 1507–1510.

(5) ——— KARRER, P., and ZUBRYS, A.
1934. WACHSTUMSVERSUCHE MIT CAROTINOIDEN. Helvetica Chim. Acta 17: 24–29, illus.

(6) GILLAM, ALBERT EDWARD, and EL RIDI, MOHAMMED SHAFIK.
1936. THE ISOMERIZATION OF CAROTENES BY CHROMATOGRAPHIC ADSORPTION. I. PSEUDO-α-CAROTENE. Biochem. Jour. 30: 1735–1742.

(7) GUILBERT, H. R.
1934. DETERMINATION OF CAROTENE AS A MEANS OF ESTIMATING THE VITAMIN A VALUE OF FORAGE. Indus. and Engin. Chem., Analyt. Ed. 6: 452–454.

(8) ———
1935. FACTORS AFFECTING THE CAROTENE CONTENT OF ALFALFA HAY AND MEAL. Jour. Nutrition 10: 45–62, illus.

(9) ——— MILLER, R. F., and HUGHES, E. H.
1937. THE MINIMUM VITAMIN A AND CAROTENE REQUIREMENT OF CATTLE, SHEEP, AND SWINE. Jour. Nutrition 13: 543–564.

(10) HART, E. B., STEENBOCK, H., HUMPHREY, G. C., and HULCE, R. S.
1924. NEW OBSERVATIONS AND A REINTERPRETATION OF OLD OBSERVATIONS ON THE NUTRITIVE VALUE OF THE WHEAT PLANT. Jour. Biol. Chem. 62: 315–322, illus.

(11) HART, G. H., and GUILBERT, H. R.
1933. VITAMIN-A DEFICIENCY AS RELATED TO REPRODUCTION IN RANGE CATTLE. Calif. Agr. Expt. Sta. Bull. 560, 30 pp., illus.

(12) HARTMAN, ARTHUR M., KANE, EDWARD A., and SHINN, LEO A.
1934. DOES THE CAROTENE IN ALFALFA HAY ACCOUNT COMPLETELY FOR ITS VITAMIN A ACTIVITY? Jour. Biol. Chem. (Amer. Soc. Biol. Chem. Proc. 28) 105 (2): xxxvi.
(13) HAUGE, S. M., WILBUR, J. W., and HILTON, J. H.
1937. A FURTHER STUDY OF THE FACTOR IN SOYBEANS AFFECTING THE VITAMIN A VALUE OF BUTTER. Jour. Dairy Sci. 20: 87–91, illus.
(14) JONES, I. R., ECKLES, C. H., and PALMER, L. S.
1926. THE RÔLE OF VITAMIN A IN THE NUTRITION OF CALVES. Jour. Dairy Sci. 9: 119–139, illus.
(15) KARRER, P., and SCHLIENTZ, W.
1934. PFLANZENFARBSTOFFE. LV. ZUM VORKOMMEN VON α- UND β-CAROTIN IN VERSCHIEDENEN NATURPRODUKTEN. Helvetica Chim. Acta 17: 7–8.
(16) KRAUSS, W. E.
1935. THE VITAMIN A CONTENT OF CORN SILAGE. Ohio Agr. Expt. Sta. Bull. 548 (Ann. Rept. 1934): 62.
(17) ——— and WASHBURN, R. G.
1936. STUDIES ON A. I. V. SILAGE. PART III—CAROTENE PRESERVATION, AND BIOLOGICAL PROPERTIES OF THE MILK. Jour. Dairy Sci. 19: 454–456.
(18) KUHN, RICHARD, and BROCKMANN, HANS.
1932. BESTIMMUNG VON CAROTINOIDEN. Hoppe-Seyler's Ztschr. Physiol. Chem. 206: 41–64, illus.
(19) ——— and BROCKMANN, HANS.
1932. HYDRIERUNGS- UND OXYDATIONSPRODUKTE DER CAROTINE ALS VORSTUFEN DES A-VITAMINS. Hoppe-Seyler's Ztschr. Physiol. Chem. 213: 1–7, illus.
(20) ——— and BROCKMANN, HANS.
1933. SEMI-β-CAROTINON; EIN VERFAHREN ZUR AUSFÜHRUNG CHEMISCHER REAKTIONEN. Ber. Deut. Chem. Gesell. 66 (Bd. 2, Abt. B): 1319–1326, illus.
(21) ——— BROCKMANN, HANS, SCHEUNERT, A., and SCHIEBLICH, M.
1933. ÜBER DIE WACHSTUMSWIRKUNG DER CAROTIN UND XANTHOPHYLLE. Hoppe-Seyler's Ztschr. Physiol. Chem. 221: 129–136.
(22) ——— and LEDERER, EDGAR.
1931. ÜBER α- UND β-CAROTIN. Hoppe-Seyler's Ztschr. Physiol. Chem. 200: 246–254.
(23) ——— WINTERSTEIN, ALFRED, and LEDERER, EDGAR.
1931. ZUR KENNTNIS DER XANTHOPHYLLE. Hoppe-Seyler's Ztschr. Physiol. Chem. 197: 141–160, illus.
(24) LIPMAN, JACOB G.
1934. AGRICULTURAL BIOCHEMISTRY. N. J. Agr. Expt. Sta. Ann. Rpt. 55: 4–7.
(25) MCCOLLUM, E. V., and SIMMONDS, NINA.
1929. THE NEWER KNOWLEDGE OF NUTRITION. Ed. 4, 594 pp., illus. New York.
(26) MCNICHOLAS, H. J.
1931. THE VISIBLE AND ULTRAVIOLET ABSORPTION SPECTRA OF CAROTIN AND XANTHOPHYLL AND THE CHANGES ACCOMPANYING OXIDATION. U. S. Bur. Standards Jour. Research 7: 171–193, illus.
(27) MEIGS, EDWARD B., and CONVERSE, H. T.
1933. SOME EFFECTS OF DIFFERENT KINDS OF HAY IN THE RATION ON THE PERFORMANCE OF DAIRY COWS. Jour. Dairy Sci. 16: 317–328.
(28) ——— and CONVERSE, H. T.
1934. VITAMIN A IN THE NUTRITION OF DAIRY CATTLE. Amer. Soc. Anim. Prod. Proc. (1933) 26: 58–61.
(29) ——— and CONVERSE, H. T.
1936. THE VITAMIN A REQUIREMENTS OF DAIRY COWS FOR REPRODUCTION AND LACTATION UNDER PRACTICAL CONDITIONS. (Abstract) Jour. Dairy Sci. 19: 438.
(30) MILLER, ELMER S.
1935. A RAPID AND ACCURATE QUANTITATIVE METHOD FOR THE DETERMINATION OF THE COMMON CAROTINOIDS; ANALYSES OF BETA-CAROTENE AND LEAF XANTHOPHYLL IN THIRTEEN PLANT TISSUES. Jour. Amer. Chem. Soc. 57: 347–349.

(31) MILLER, ELMER S.
1935. IMPROVED METHODS FOR THE PURIFICATION OF THE COMMON CAROTENOIDS, AND THE QUANTITATIVE MEASUREMENT OF THEIR ABSORPTION SPECTRA. Bot. Gaz. 96: 447–467, illus.
(32) MOORE, L. A.
1937. TECHNIQUE USED IN STUDYING VITAMIN A REQUIREMENTS OF DAIRY CATTLE. Jour. Dairy Sci. 20: 427.
(33) NICKERSON, DOROTHY.
1929. A METHOD FOR DETERMINING THE COLOR OF AGRICULTURAL PRODUCTS. U. S. Dept. Agr. Tech. Bull. 154, 32 pp., illus.
(34) PETERSON, W. H., BOHSTEDT, G., BIRD, H. R., and BEESON, W. M.
1935. THE PREPARATION AND NUTRITIVE VALUE OF A. I. V. SILAGE FOR DAIRY COWS. Jour. Dairy Sci. 18: 63–78, illus.
(35) ROGERS, L. A.
1936. NUTRITION OF DAIRY COWS. U. S. Bur. Dairy Indus. Ann. Rept. 1936: 3–4.
(36) RUSSELL, WALTER C.
1929. THE EFFECT OF THE CURING PROCESS UPON THE VITAMIN A AND D CONTENT OF ALFALFA. Jour. Biol. Chem. 85: 289–297.
(37) ——— TAYLOR, M. W., and CHICHESTER, D. F.
1934. THE EFFECT OF THE CURING PROCESS UPON THE CAROTENE AND VITAMIN A CONTENT OF ALFALFA. N. J. Agr. Expt. Sta. Bull. 560, 8 pp., illus.
(38) SCHERTZ, F. M.
1928. THE EXTRACTION AND SEPARATION OF CHLOROPHYLL ($\alpha+\beta$) CAROTIN AND XANTHOPHYLL IN FRESH GREEN LEAVES, PRELIMINARY TO THEIR QUANTITATIVE DETERMINATION. Plant Physiol. 3: 211–216.
(39) SHEPHERD, J. B., WOODWARD, T. E., and GRAVES, R. R.
1936. EFFECT OF MOISTURE CONTENT AND DENSITY OF STORED ROUGHAGE ON TEMPERATURES ATTAINED DURING STORAGE AND THE QUALITY OF THE PRODUCT. Jour. Dairy Sci. 19: 467–468.
(40) SMITH, JAMES H. C.
1936. CAROTENE. X. A COMPARISON OF ABSORPTION-SPECTRUM MEASUREMENTS ON α-CAROTENE, β-CAROTENE AND LYCOPENE. Jour. Amer. Chem. Soc. 58: 247–255, illus.
(41) ——— and MILNER, HAROLD W.
1934. CAROTENE. VII. PHYSICAL PROPERTIES OF CAROTENES FROM DIFFERENT PLANT SOURCES. Jour. Biol. Chem. 104: 437–447, illus.
(42) STRAIN, Harold H.
1934. CAROTENE. VIII. SEPARATION OF CAROTENES BY ADSORPTION. Jour. Biol. Chem. 105: 523–535.
(43) UNITED STATES BUREAU OF AGRICULTURAL ECONOMICS.
1936. HANDBOOK OF OFFICIAL HAY STANDARDS. 62 pp.
(44) WILLSTÄTTER, RICHARD, and STOLL, ARTHUR.
1928. INVESTIGATIONS OF CHLOROPHYLL; METHODS AND RESULTS. Auth. English trans. from the German, by Frank Milton Schertz, and Albert R. Merz. 385 pp., illus. Lancaster, Pa.
(45) WISEMAN, HERBERT G., and KANE, EDWARD A.
1936. THE DETERMINATION OF CAROTENE IN FRESH PLANT MATERIALS. Jour. Biol. Chem. (Amer. Soc. Biol. Chem. Proc. 30) 114 (1): cviii.
(46) ——— KANE, EDWARD A., and CARY, C. A.
1936. RATE OF DECOMPOSITION OF CAROTENE IN HAYS DURING STORAGE AT DIFFERENT SEASONS OF THE YEAR. Jour. Dairy Sci. 19: 466–467.

HOST-PARASITE RELATIONS IN RED CLOVER PLANTS RESISTANT AND SUSCEPTIBLE TO POWDERY MILDEW, ERYSIPHE POLYGONI[1]

By OLIVER F. SMITH[2]

Assistant agronomist, Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

Plants of red clover, *Trifolium pratense* L., show wide differences in susceptibility to the powdery mildew fungus, *Erysiphe polygoni* DC. Most plants are highly susceptible and the fungus develops profusely, producing an abundance of superficial mycelium and conidia. There are, however, occasional plants on which there is no development of the fungus beyond spore germination and penetration into the host. Between these two extremes are plants upon which the fungus develops with different degrees of severity but which do not lend themselves to a rigid classification because of the many gradations that exist between susceptibility and apparent immunity.

In papers on the powdery mildews little has been recorded on the interaction between host and parasite in resistant and susceptible plants within a species. Smith (*8*)[3] and Hirata (*1*) have described in some detail haustorial development of several powdery mildew fungi but only on congenial host plants. Salmon (*7*) and Neger (*2*) have described the behavior of powdery mildew fungi on noncongenial host plants, but in their work plants of one species were inoculated with mildew from plants of other species.

The object of these investigations was to obtain information on the nature of the interaction between the host *Trifolium pratense* and the parasite *Erysiphe polygoni* that would be of assistance in breeding red clover for mildew resistance. The different degrees of superficial development of the fungus on the host are obviously associated with a host-parasite relation that can be examined only by cytological methods. Therefore cytological studies have been made on the penetration of the fungus into the host and the fate of the infecting hyphae in the epidermal cells of plants showing different degrees of susceptibility or resistance. This work parallels some of that done on the rusts, but since the many papers dealing with host-parasite relations in that group of diseases have been comprehensively reviewed elsewhere (*5*, *6*) it seems needless to consider them here except as they have a bearing on these investigations.

MATERIALS AND METHODS

Plants selected for these studies were for convenience arbitrarily placed in three classes, namely, susceptible, moderately resistant,

[1] Received for publication June 2, 1938; issued November 1938. Cooperative investigations of the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Wisconsin Agricultural Experiment Station.

[2] The writer wishes to express his appreciation to Eugene Herrling, assistant in plant pathology, Wisconsin Agricultural Experiment Station, for preparing the photographs.

[3] Italic numbers in parentheses refer to Literature Cited, p. 682.

and highly resistant, according to the amount of mildew that developed on them as shown in figure 1.

On susceptible plants, the fungus developed profusely and produced an abundance of mycelium over the surface of the leaves. The plants classified as moderately resistant occupied positions between the extremes of susceptibility and resistance, and consequently the amount of fungus development on them differed considerably. On vigorously growing leaves of highly resistant plants there was no fungus development beyond spore germination and penetration into the underlying epidermal cell. On some plants in this class, mildew developed slightly on senescent leaves but a few individuals were observed on which no mildew developed at any stage of their life; the latter were considered immune. Highly resistant plants are quite rare and therefore studies on that class were restricted to a few individuals, whereas in the

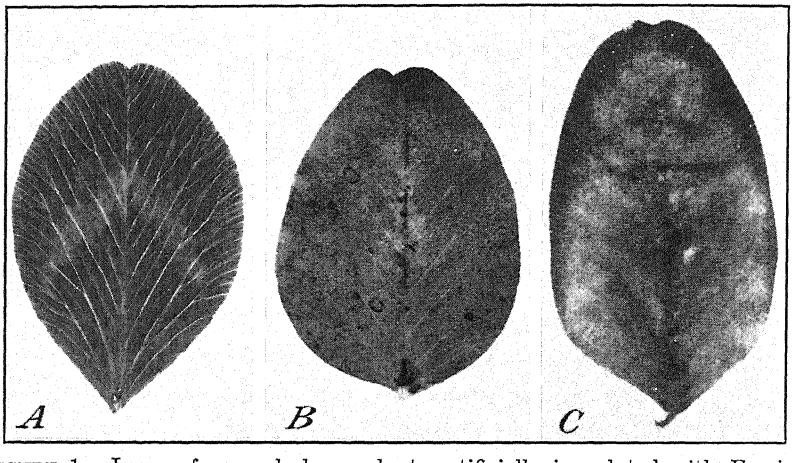


FIGURE 1.—Leaves from red clover plants artificially inoculated with *Erysiphe polygoni: A*, Leaf from a highly resistant plant, showing no fungus development; *B*, leaf from a moderately resistant plant, showing a moderate amount of fungus development and also a necrosis of leaf tissue following establishment of the parasite; *C*, leaf from a susceptible plant, showing abundant fungus development. × about 1½.

susceptible and moderately resistant classes samples of infected leaves were taken from a large number of plants. In all the work reported here, only young, vigorously growing leaves were used.

The plants were grown in the greenhouse and inoculated by brushing spores from mildewed leaves onto uninfected ones with a camel's-hair brush. Inoculations were made during March and April when abundant sunlight and a suitable greenhouse temperature, seldom exceeding 85° F., favored mildew development.

After inoculation, samples of leaf tissue were collected at 3-hour intervals for a period of 24 hours, then at 24-hour intervals for the next 3 days, and every fourth day thereafter for the following 2 weeks, and were fixed for 24 hours in Karpechenko's modification of Navashin's fixing fluid, consisting of equal parts of solution A (195 cc of water, 30 cc of glacial acetic acid, and 3 g of chromic acid) and solution B (195 cc of water, 30 cc of formalin (37 percent formaldehyde))

The process of dehydration and infiltration with paraffin was done according to the schedule given by Rawlins (*4, p. 21*), by the use of cedar oil following a series of alcohols of various concentrations up to 95 percent. Sections were cut 6μ and 8μ in thickness. Staining with safranine and fast green gave satisfactory differentiation between host and parasite. Sudan IV and ruthenium red were used for staining the cuticle and cell walls.

A modification of the cleared-leaf method described by Peace (*3*) was used for observing spore germination and early stages of infection on the leaves. Bits of leaf tissue were fixed in equal parts of alcohol and acetic acid until all the green pigments were removed; they were then stained in acid fuchsin in lactophenol, rinsed in alcohol-acetic acid (1:9), and cleared in chloral hydrate for examination.

Counts of spore germination on leaf tissue were made with the aid of an Ultrapak microscope.

Since physiologic forms of *Erysiphe polygoni* on red clover have been reported (*9*) questions may arise regarding the purity of the mildew used. Unfortunately the plants by which these physiologic forms were differentiated no longer exist. The plants used for these studies were consistent in the amount of mildew that developed on them and in host-parasite relations, and it is therefore assumed that physiologic forms did not confuse the results.

SPORE GERMINATION AND EARLY STAGES OF INFECTION

Observations have shown that there are no differences in percentage of spore germination or in the manner in which spores germinate, form appressoria, and effect penetration on the three classes of plants. Therefore the description of these processes applies to plants in any of the three classes.

On susceptible plants 600 conidia out of 691 observed had germinated in 20 hours after inoculation; on moderately resistant plants 472 had germinated out of 575 observed, and on highly resistant plants 618 had germinated out of 698 observed. The percentage germination on the three classes of plants was, therefore, 86, 82, and 88, respectively.

Gross observations on spore germination and on early stages of infection were readily obtained on cleared leaves stained with acid fuchsin. Under favorable conditions spores begin to germinate about 3 or 4 hours after being freed from conidiophores and coming to rest on the surface of a leaf. A germ tube grows out from one end of the spore and forms a well-defined appressorium (fig. 2, *A* and *E*), which becomes firmly attached to the leaf surface. Subsequently an infection hypha enters the underlying cell and an infection pore is apparent at the point of penetration (fig. 2, *B* and *F*). The appressoria are of various shapes and sizes and may form anywhere on the surface of an epidermal cell, but are most frequently located at the edge of a cell or over the walls which separate these cells. They sometimes form over stomata, but infection hyphae have never been observed to enter through these openings. A septum is formed between the appressorium and the spore (fig. 2, *C*). Under favorable conditions a spore germinates, forms an appressorium, and effects penetration in about 5 to 7 hours.

Detailed observations on penetration were made on microtome-sectioned leaf tissue. After an appressorium is attached firmly to the surface of the leaf a slender infection hypha begins to penetrate the cuticle and outer cell wall. During the very early stages of penetration there is an apparent swelling of the subcuticular materials for some distance around the point of penetration, which causes a separation of the cuticle from the cell wall. Figure 3, *A* and *F*, page 676 shows

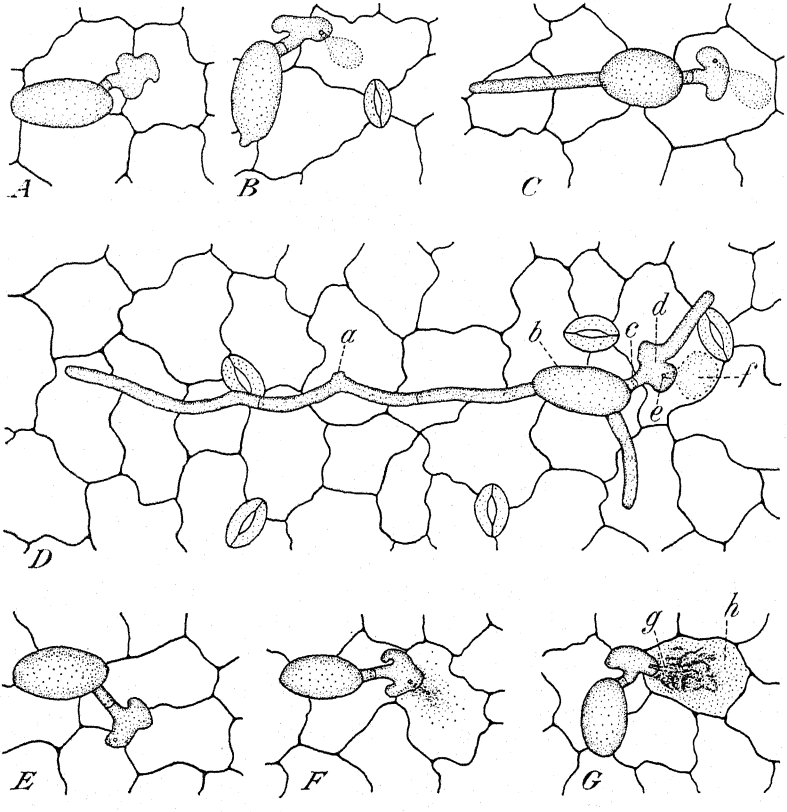


FIGURE 2.—Camera-lucida sketches of spore germination and early stages of infection by *Erysiphe polygoni* from cleared leaves of red clover: *A*, *B*, *C*, and *D*, On a susceptible plant; *E*, *F*, and *G*, on a resistant plant. *a*, Young appressorium from which penetration probably would have taken place into the underlying epidermal cell; *b*, spore; *c*, germ tube; *d*, appressorium; *e*, infection pore; *f*, haustorium and surrounding sheath; *g*, infection peg in (*h*) necrotic cell. × about 385.

infection hyphae that have pierced the cuticle and are in the swollen subcuticular area. There is no apparent alteration of the cuticle. Other stages of infection are shown in figure 3, *B* and *G*, where the infection hyphae have passed through the subcuticular region, entered the cell wall, and are somewhat enlarged at their distal ends.

Concurrent with these early stages of penetration, an ingrowth from the cell wall, which appears to be an addition of new materials

to its inner surface, develops at each point of penetration. In some cases the ingrowth extends over a third of the distance across the infected cell; in others it is barely perceptible. The amount produced is usually a little less than that shown in figure 4, *C* page 678. While an infection hypha is still within the cell wall and the ingrowth, a halo area (fig. 4, *B*) is often visible immediately around its tip, which indicates that these materials have been dissolved by the fungus as it penetrated into the cell. The plasma membrane passes up the sides and over the end of this structure and is later invaginated by the invading fungus.

INFECTION OF SUSCEPTIBLE PLANTS

An infection hypha, after making its way through the ingrowth of the cell wall, develops into a simple haustorium. This absorption organ consists of a slender neck, which passes through the wall of the epidermal cell and enlarges into a vesicular, distal portion, the ingrowth of the wall forming a collar which surrounds and accompanies the neck for some distance into the cell (fig. 3, *D* and *E*). The haustorium is uniseptate, uninucleate, and surrounded by a well-defined wall.

Around the haustorium is a conspicuous thick sheath containing a considerable amount of dark-staining, somewhat granular material, which is frequently gathered into irregular masses varying in extent and outline. This material often makes it difficult to see the septum in the haustorium. No evidence was obtained concerning the nature of this granular substance.

Surrounding the haustorium sheath is a conspicuous membrane which in early stages sometimes appears to be connected with the ingrowth of the cell wall, but in later stages becomes separated from the ingrowth and thus establishes itself as being independent of that structure (fig. 3, *E*). It is difficult to determine the exact nature of this sheath membrane. In early stages it is very thin and undoubtedly consists largely of plasma membrane, but in older stages it appears too thick to consist of plasma membrane alone, as suggested by Smith (*8*), unless the plasma membrane has become decidedly thickened along that portion surrounding the sheath. It appears more likely to have been produced by the plasma membrane of the host. This is in agreement with observations of a number of other investigators whose works have been reviewed by Rice (*5*, *6*).

The host cell nucleus moves toward the invading fungus. While stained slides were being examined for successive stages in the development of haustoria it was noted that the host nucleus was nearly always located near the invading fungus and had in all probability migrated to that position. In entire leaf tissue which was cleared and stained the host nucleus was never located away from the haustorium sheath, whether the haustorium was at the center of the cell or at one side of it. This is apparently the usual relationship between host nucleus and fungus haustorium, as Rice (*5*) lists 23 cases of habitual contact against 9 cases of occasional contact and 3 negative cases.

On susceptible plants there is no apparent antagonistic reaction between host and parasite. The fungus develops rapidly, and the contents of the invaded host cell appear to be as well organized as the contents of noninfected ones.

On a susceptible host, a spore germinates, forms an appressorium, penetrates into the host, and develops a haustorium in about 9 hours after inoculation (fig. 2, *B*). Another hypha then grows out from the other end of the spore (fig. 2, *C*) and develops into branching

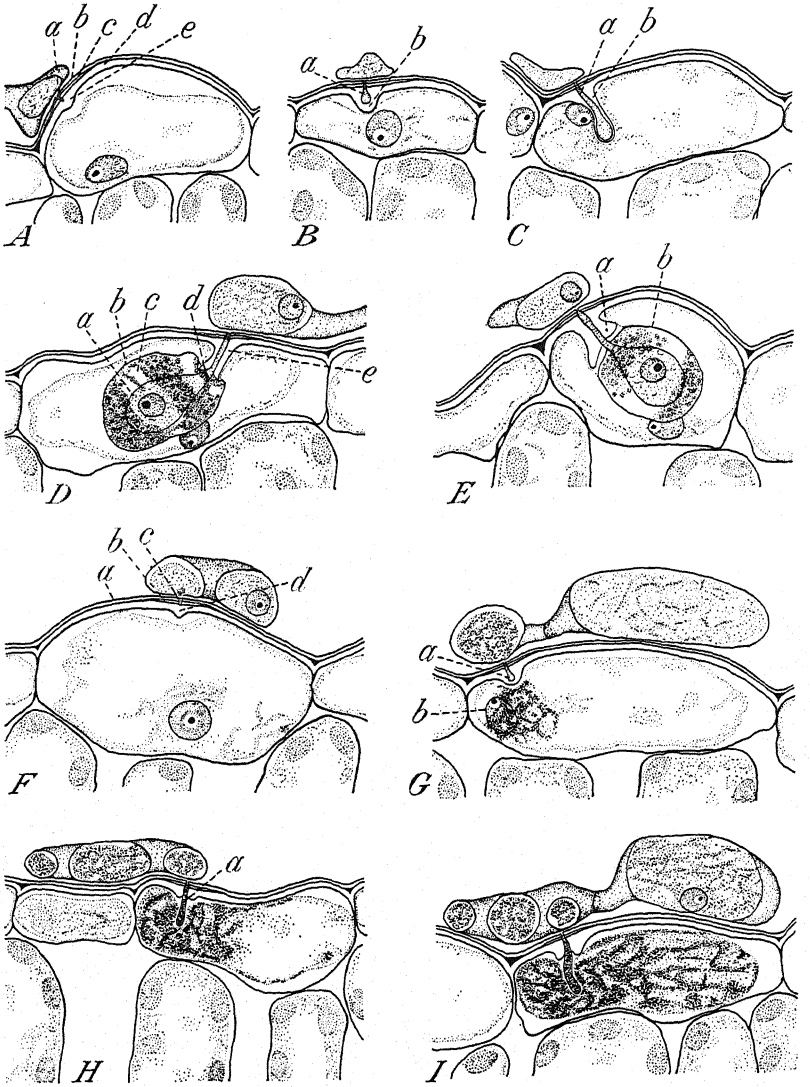


FIGURE 3.—FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

mycelium which spreads over the surface of the leaf, forming appressoria and developing haustoria in the epidermal cells of the host. Additional mycelium may also develop from the spore and in some cases from the first-formed appressorium. Figure 2, *D*, shows an 18-hour-old infection where the spore has formed a haustorium in

another appressorium at *a*, where penetration into the underlying cell with the formation of a haustorium is expected. The mycelium continues in this fashion to spread over the surface of the leaf, forming appressoria and haustoria and eventually conidiophores and conidia. Sporulation begins about 5 or 6 days after inoculation. Leaves of a congenial host plant will support a heavy growth of mildew for about 3 weeks, after which the leaves gradually become irregularly chlorotic and die.

INFECTION OF HIGHLY RESISTANT PLANTS

On a highly resistant host the parasite seldom develops further than the formation of an appressorium and the production of an infection hypha (fig. 2, *G*). The germ pore is formed on the lower side of the appressorium and an infection hypha enters the underlying epidermal cell as shown in figures 2 and 3. As the infection hypha enters the host cell there is a decidedly antagonistic reaction between host and parasite. The cytoplasm of the host stains heavily and appears to have undergone some degree of disorganization around the point of infection. In some instances this reaction is in evidence even before penetration into the cell is complete. Figure 3, *G*, shows an early stage of infection where the cell contents stained heavily before the fungus had actually entered the cell. Because of the small size of the young infection hypha it is impossible to determine whether there is a similar effect on the parasite at this stage of development but, if so, it is not severe enough to stop the progress of the fungus. As the infection hypha grows into the cell protoplast, apparently by an invagination of the plasma membrane, the antagonistic reaction becomes more intense (fig. 3, *H*) and within 48 hours after inoculation the entire contents of the cell and the infection hypha become disorganized masses of dark-staining granular materials (fig. 3, *I*) and the infected cell, as well as the spore and appressorium, collapses (fig. 4, *G* and *H*). An infection hypha seldom develops into more than a slender infection peg (fig. 4, *F*), although a few instances have been ob-

EXPLANATORY LEGEND FOR FIGURE 3

FIGURE 3.—Camera-lucida sketches of infection of epidermal cells of leaves of red clover by *Erysiphe polygoni: A, B, C, D,* and *E,* On susceptible plant; *F, G, H,* and *I,* on resistant plant. × about 930.

A.—Infection hypha (*a*) has pierced cuticle (*b*) and swollen subcuticular area (*c*) and entered cell wall (*d*). Note incipient ingrowth of cell wall at *e*.

B.—Infection hypha (*a*) has entered ingrowth of cell wall (*b*) and is slightly enlarged at its distal end. Nucleus of host cell is near point of penetration.

C.—Infection hypha (*a*) has passed nearly through ingrowth of cell wall (*b*).

D.—Fully developed haustorium (*a*) with sheath membrane (*b*), sheath (*c*), and septum (*d*). Ingrowth of cell wall (*e*) forms collar around neck of haustorium. Host penetration and development of haustorium are accomplished in about 9 hours after inoculation.

E.—Infection about 12 days old, showing haustorium sheath membrane (*b*) separated from ingrowth of cell wall (*a*). Note granular material in haustorium sheath in *D* and *E*.

F.—Infection hypha (*c*) has pierced cuticle (*a*) and is nearly through swollen subcuticular area (*b*). Note incipient ingrowth of cell wall at *d*.

G.—Infection hypha (*a*) is nearly through ingrowth of cell wall, and host cytoplasm has stained deeply near point of penetration. Host nucleus (*b*) is near point of penetration.

H.—Infection hypha (*a*), which stained heavily, has entered cell and is surrounded by heavily stained granular materials of host cell.

I.—Infection approximately 36 hours old. Infection hypha and cell contents [illegible] granular, have stained heavily, and are apparently dead.

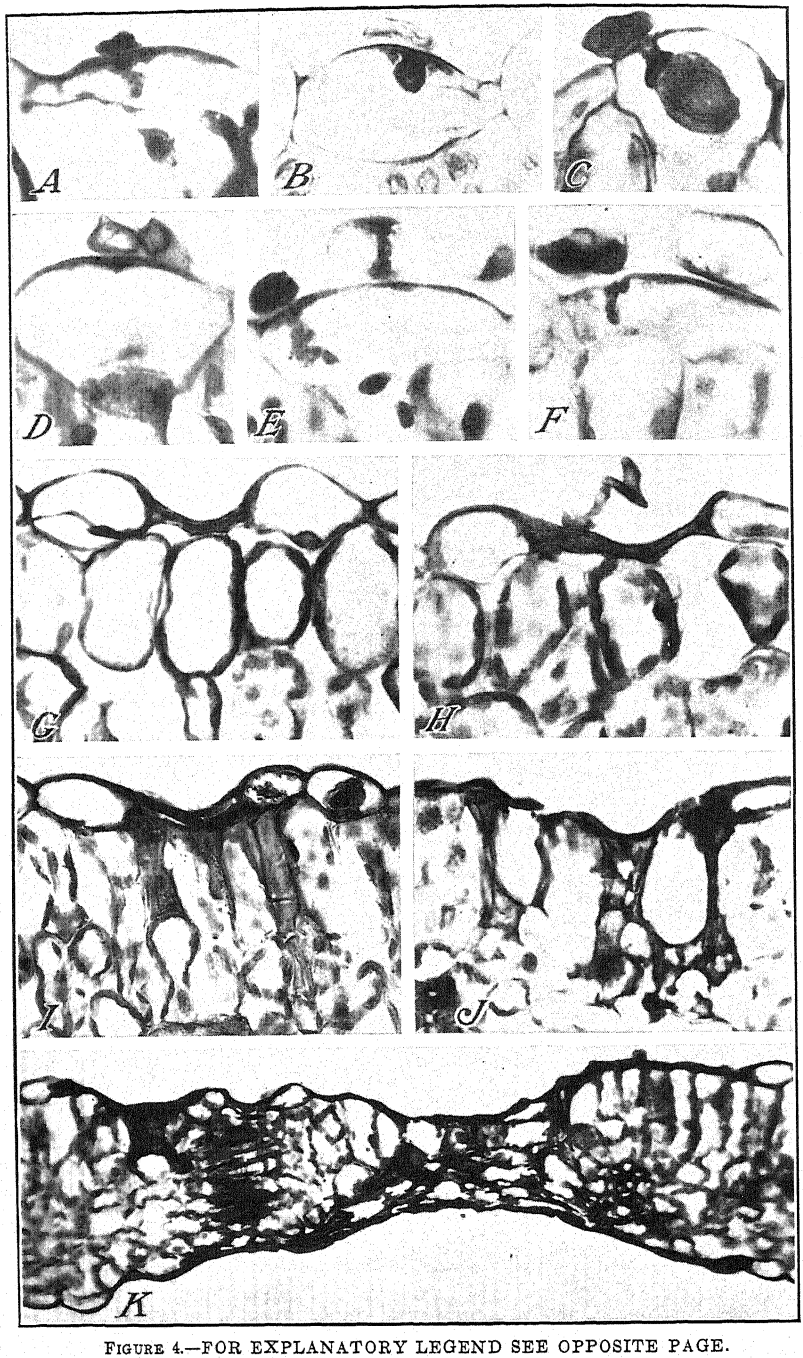


FIGURE 4.—FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

served in which the infection hypha had become slightly enlarged at its distal end before the progress of the fungus was checked. Working with certain biological forms of *Erysiphe* and resistant hosts, Salmon (*7, p. 220*), found that incipient haustoria were sometimes formed but were—

> arrested and gradually disorganized under the influences at work in the cell of the "wrong" host-plant, or if the haustorium attains its full size it is hindered by these influences from carrying on its normal functions, and thus supplying to the fungus the food necessary for the production of mycelium, etc.

Injury to cells surrounding the infected one differs with individual plants. In some cases only the infected cell becomes necrotic, with no apparent adverse effects on the surrounding cells (fig. 4, *G* and *H*). In other cases cells adjacent to the infected one are killed and small brown necrotic areas are produced which are readily observed with the aid of an Ultrapak microscope but which cannot be seen macroscopically. A few plants have been observed in which sufficient cells became necrotic around each point of infection to produce macroscopically visible flecks on the leaves. It seems that in plants in which only the infected cell is killed, the host-cell contents become coagulated before the effects of the antagonistic interaction between host and parasite are transmitted to the surrounding cells. In plants that show death of a few cells in addition to the infected one, the effects of the host-parasite interaction are probably transmitted to those cells and cause their death. Whatever factor or factors may cause this antagonistic reaction, they are severe with highly resistant plants, less severe with plants possessing an intermediate degree of resistance, and apparently lacking with highly susceptible plants. Cytological investigations have not revealed their identity.

In resistant plants there is evidence also that the host cell nucleus moves toward the invading hypha. It was difficult to identify the

EXPLANATORY LEGEND FOR FIGURE 4

FIGURE 4.—Photomicrographs of different stages of infection on leaves of red clover plants by *Erysiphe polygoni*.

A, Early stage of infection on susceptible plant, showing infection hypha in ingrowth of cell wall. × about 880.

B, Early stage of infection on susceptible host, showing halo region around end of infection hypha in ingrowth of cell wall. × about 880.

C, Fully developed haustorium, with its sheath and sheath membrane, in epidermal cell of susceptible plant; × about 880.

D, Early stage of infection on resistant plant, showing very early development of ingrowth of cell wall and swelling of subcuticular materials which has caused a separation of cuticle from cell wall around point of penetration. Infection hypha has pierced cuticle and is in swollen subcuticular area. × about 880.

E, Young infection on resistant plant, showing dark-stained cytoplasm immediately below infection hypha, which is still in ingrowth of cell wall. × about 880.

F, Infection approximately 36 hours old on resistant plant. Infection hypha has entered host cell but has only developed into a slender peg. Both infection hypha and cell contents are granular and have stained heavily. × about 880.

G, Infection 7 days old on highly resistant plant. Infected cell has collapsed; no apparent injury to surrounding cells; × about 590.

H, Infection 20 days old on highly resistant plant. Infected cell has collapsed; no apparent injury to surrounding cells. × about 590.

I, *J*, and *K*, Infections 8, 12, and 18 days old, respectively, on moderately resistant plant that showed extensive necrosis of leaf tissue following infection. *I*, Infected cell has collapsed; underlying palisade cells are necrotic. Infected cell at right had not collapsed when this material was fixed. × about 400. *J*, Several epidermal and palisade cells have collapsed. × about 400. *K*, Necrosis and collapse of cells has occurred through leaf. × about 250.

host nucleus after the early stages of infection because of the disorganization of the host protoplasm, but when identifiable it was near the invading fungus.

INFECTION OF MODERATELY RESISTANT PLANTS

Between the extremes of susceptibility and resistance are plants upon which the fungus develops with different degrees of severity but which cannot be rigidly classified because of the many gradations that exist. Furthermore, experience has shown that when light is abundant the development of mildew on these plants increases, but with decreased light there is a smaller amount of mildew. However, the plants considered here as showing an intermediate or moderate degree of resistance did so under the seemingly optimum conditions for mildew development in which the highly resistant and highly susceptible plants were studied. On plants approaching a high degree of resistance, growth of the fungus is barely perceptible to the unaided eye and very few conidia are produced. On plants of greater susceptibility, growth of the fungus increases and there is also an increase in the number of conidia produced. Sectioned leaves of these plants showed that haustoria are produced in the epidermal cells, but on the more resistant plants they are not as large as those in epidermal cells of highly susceptible plants. With the exception of one plant, which will be considered later, there was little indication of adverse effects from host-parasite reaction on infected host cells except on plants approaching a high degree of resistance. In these cases the cytoplasm stained a little darker than in susceptible plants and appeared to be granular and somewhat disorganized.

One plant was observed that showed a distinct type of host reaction (fig. 1, *B*), but because mildew developed on it only in moderate amounts it is considered here with the class of moderately resistant plants. When this plant was inoculated, the fungus became established in a manner similar to that described for susceptible plants, and maintained a seemingly congenial relationship with the host plant for a short period. A considerable amount of mycelium and many spores were produced, sporulation beginning about 6 days after inoculation. After about 8 days, small brown necrotic spots developed on the leaves. At their earliest stages of development these spots involved only the infected epidermal cell and the underlying palisade cells (fig. 4, *I*). These spots increased in size quite rapidly, and about 12 days after infection several epidermal and palisade cells collapsed (fig. 4, *J*). Eighteen days after infection these necrotic areas extended entirely through the leaf (fig. 4, *K*), and because of increase in size they coalesced, and the death of the leaf soon followed. There can be little doubt that infection of epidermal cells initiated the development of these spots, as leaves of this plant kept free of inoculum showed no signs of necrosis.

Sectioned leaves of this plant showed that the collapse of cells was preceded by the deposition of a yellowish substance chiefly along the walls, first in the infected epidermal cell, then in the surrounding cells. By the time the infected epidermal cell had collapsed, the deposition of this substance and cell necrosis had extended into the underlying palisade cells (fig. 4, *I*). Necrosis and collapse of palisade and spongy parenchyma cells continued to spread throughout the

tissues in the vicinity of the infected cell (fig. 4, *J*) and eventually the necrotic area extended throughout the leaf (fig. 4, *K*). It appears that the antagonistic reaction between host and parasite was not severe enough to cause immediate death of either and that during the prolonged interaction an opportunity was afforded for the effects of this interaction to be transmitted to surrounding cells, causing reactions within them which eventually brought about their death.

SUMMARY

Results are reported of cytological investigations on infection and development of *Erysiphe polygoni* on susceptible, moderately resistant, and highly resistant red clover plants.

Early stages of infection were the same on the three classes of plants. Spores germinated, formed appressoria, and penetrated directly into epidermal cells of the host leaf. Concurrent with the early stages of penetration, an ingrowth of the wall developed at each point of penetration.

On susceptible plants an infection hypha, after entering a cell, developed into a simple haustorium. This absorption organ consisted of a slender neck that passed through the wall of the epidermal cell and enlarged into a vesicular distal portion, the ingrowth of the cell wall forming a collar that surrounded and accompanied the neck for some distance into the cell. The haustorium was surrounded by a thick sheath containing a considerable amount of dark-staining, somewhat granular material. This sheath was in turn surrounded by a conspicuous membrane.

On highly resistant hosts, an infection hypha entered an underlying epidermal cell, but its progress was soon stopped because of an antagonistic reaction between host and parasite. The cytoplasm of the host stained heavily and appeared to undergo some degree of disorganization around the point of infection, in some cases even before penetration into the cell was complete. As an infection hypha grew into the cell protoplast the antagonistic reaction became more intense, and finally the entire contents of the cell, as well as the infection hypha, became disorganized masses of dark-staining material and the infected cell ultimately collapsed. In some plants only the infected cell became necrotic, with no apparent adverse effects on the surrounding cells. With other plants, however, cells adjacent to the infected one became discolored and small brown necrotic areas were produced.

On moderately resistant plants, the fungus established itself in much the same manner as on highly susceptible ones and was able to maintain what appeared to be a fairly congenial relationship with the host plants. Growth on the surface of the plant varied with individuals, being roughly in proportion to the degree of susceptibility possessed by the plant. One plant was observed that showed an extensive necrosis of leaf tissue following infection. The fungus established itself and maintained a seemingly congenial relationship with the host for a period of about 8 days, after which necrotic spots appeared on the leaves. These increased in size, coalesced, and eventually killed the leaf.

The host nucleus moved toward the invading fungus in both resistant and susceptible plants.

LITERATURE CITED

(1) HIRATA, K.
1937. THE HAUSTORIUM OF SOME POWDERY MILDEWS. Ann. Phytopath. Soc. Japan 6: 319–334, illus. [In Japanese. English summary, pp. 333–334.]

(2) NEGER, F. W.
1923. BEITRÄGE ZUR BIOLOGIE DER ERYSIPHEEN. III. DER PARASITISMUS DER MEHLTAUPILZE—EINE ART VON GEDULDETER SYMBIOSE. Flora [Jena] 116: 331–335, illus.

(3) PEACE, L. M.
1910. NOTES UPON THE CLEARING AND STAINING OF LEAVES AND STEMS. Plant World 13: 93–96.

(4) RAWLINS, THOMAS ELSWORTH.
1933. PHYTOPATHOLOGICAL AND BOTANICAL RESEARCH METHODS. 156 pp., illus. New York and London.

(5) RICE, MABEL AGNES.
1927. THE HAUSTORIA OF CERTAIN RUSTS AND THE RELATION BETWEEN HOST AND PATHOGENE. Bull. Torrey Bot. Club 54: 63–153, illus.

(6) ———
1935. THE CYTOLOGY OF HOST-PARASITE RELATIONS. Bot. Rev. 1: 327–354, illus.

(7) SALMON, ERNEST S.
1905. ON THE STAGES OF DEVELOPMENT REACHED BY CERTAIN BIOLOGIC FORMS OF ERYSIPHE IN CASES OF NON-INFECTION. New Phytol. 4: 217–222, illus.

(8) SMITH, GRANT.
1900. THE HAUSTORIA OF THE ERYSIPHEÆ. Bot. Gaz. 29: 153–184, illus.

(9) YARWOOD, CECIL E.
1936. HOST RANGE AND PHYSIOLOGIC SPECIALIZATION OF RED CLOVER POWDERY MILDEW, ERYSIPHE POLYGONI. Jour. Agr. Research 52: 659–665, illus.

DIFFERENTIATION OF WOOD-DECAYING FUNGI BY THEIR REACTIONS ON GALLIC OR TANNIC ACID MEDIUM [1]

By Ross W. Davidson, *associate mycologist*, W. A. Campbell, *assistant pathologist*, and Dorothy J. Blaisdell, *junior pathologist*, *Division of Forest Pathology*,[2] *Bureau of Plant Industry*, *United States Department of Agriculture*

INTRODUCTION

In a study of only a few wood-decaying fungi Bavendamm [3] found that those species causing white decay formed a dark diffusion zone or "corona" under the fungus mats when grown on media containing small amounts of gallic or tannic acid, whereas those causing brown checked decay gave no such reaction. The brown diffusion zone was considered to be the result of oxidation of the acids, and hereafter this reaction will be referred to as "oxidase" reaction. Bavendamm also pointed out the effect of various concentrations of the acids on growth of the several species and suggested the use of such reactions for identification purposes should they be found consistent for a greater number of species.

Campbell [4] recorded the oxidase reactions of species of *Fomes* on tannic acid medium and found them to be consistent with Bavendamm's prediction.

It is the purpose of the present paper to give results of repeated tests of a greater number of species, representing several families and numerous genera, mostly of the Hymenomycetes. The above-mentioned tests have been used to assist in developing a method of identifying pure cultures of the wood-decaying fungi. Many such fungi have been isolated from decay in living trees on which no sporophores occurred. Such a test as the oxidase reaction, which separates the species into two distinct groups, is, when combined with numerous macroscopic and microscopic characters, very useful. The fact that the media used for the oxidase test also have a toxic effect on some species and not on others makes them of even greater value for identification purposes.

Growth rates, as well as oxidase reactions, of the fungi have been recorded on media of only one concentration, namely 0.5 percent of gallic or tannic acid added to plain malt agar as recommended by Bavendamm, although it is realized that other concentrations might give additional information of value.

[1] Received for publication June 9, 1938; issued November 1938.

[2] In cooperation with the Civilian Conservation Corps. The authors express their appreciation to Dr. L. O. Overholts, of the Pennsylvania State College and Agricultural Experiment Station, for his interest and cooperation.

[3] Bavendamm, W. Über das vorkommen und den nachweis von oxydasen bei holzzerstörenden pilzen. Zuschr. Pflanzenkrank. u. Pflanzenschutz 38: [257]–276, illus. 1928.

[4] Campbell, W. A. The cultural characteristics of the species of fomes. Bull. Torrey Bot. Club 65: 31–69, illus. 1938.

SOURCE OF CULTURES

The cultures of the different fungi, the majority of which are wood-decaying, used to determine the oxidase reaction of the various species, were those previously identified and maintained in a collection by the Division of Forest Pathology.[5] Most of the isolates were from tissue of sporophores that had been carefully identified, many of them by L. O. Overholts, or from incipient decay with which identifiable sporophores were associated. Since isolates from these two sources react alike in culture, no indication is given in the list as to what proportion of the cultures used for each species were from sporophores. A few may not be definite wood decayers, but since they were associated with dead or decaying wood, they are included to make the study as complete as possible.

METHODS

The oxidase test is made by growing the fungus to be tested on malt agar to which gallic or tannic acid has been added. Gallic acid medium was prepared by the addition of 0.5 percent of gallic acid (Mallinckrodt, U. S. P. crystallized) to malt agar (1.5 percent of Difco malt with 2 percent of agar). Twenty grams of powdered agar and 15 g of Difco malt were dissolved in 850 cc of water in a 2-liter flask. One hundred and fifty cubic centimeters of water was placed in a separate flask. The dissolved malt agar and the flask of water were autoclaved for 20 minutes at a pressure of 15 pounds. Five grams of gallic acid was dissolved in the flask of sterilized water after removal from the autoclave. Heating gallic or tannic acid with agar causes hydrolysis of the agar; therefore the two cannot be autoclaved together. When the malt agar had cooled so that it could be handled without burning the hands, the gallic acid solution was added and thoroughly mixed. The resulting gallic acid medium was then quickly poured into 9-cm Petri dishes, about 35 cc to each dish. The dishes were spread in a single layer to insure rapid cooling. Gallic acid does not change the color of malt agar. Tannic acid medium was prepared in a similar manner, using 0.5-percent tannic acid (Mallinckrodt, U. S. P., powdered). This medium has a milk-white appearance.

In order to determine the effect of old and young mycelium on gallic and tannic acid media, two series of tests were made in which 1-year-old cultures and 2-month-old cultures, respectively, were used. Cultures of these different ages were used to see whether the older cultures had accumulated oxidases, which might produce stronger reactions. Results indicated that age of cultures beyond 2 months made little difference in reaction or rate of growth, as long as the cultures were still vigorous. Reactions are reported as averages, while in the case of growth the usual range in diameter of mat is given.

Gallic and tannic acids appear toxic to many fungi, and conclusive results seemed to depend upon the growth of the fungus on the media. For this reason large pieces of inoculum, 4 to 6 mm square, with as

[5] A number of the cultures in the reference collection were contributed by L. O. Overholts, Irene Mounce, Clyde Christensen, and Ray R. Hirt.

much fungus material as possible, were placed, mycelium down, on the agar and pressed gently in order to make close contact. When working with slow-growing fungi, from one to three inoculations of the same species were made in a single dish. Several commercial brands of gallic and tannic acids were tested to determine whether different sources of these acids would give comparable results. In general, the oxidase reactions were consistent but the growth rates varied considerably. Because of evident differences in commercial gallic and tannic acids, when comparing growth and to a lesser extent oxidase reactions of different fungi, it is important to use acids from the same commercial sources.

All the fungi, with the exception of a few recent acquisitions were tested for the oxidase reaction at least three times over a period of 2 years. The reactions and, to a lesser extent, growth (table 1) have appeared relatively consistent.

Observations upon the intensity of reaction and growth of mycelium were made at the end of 7 days for cultures kept in diffused light at room temperature (about 25° C.). In doubtful cases the cultures were kept for an additional 7 days. Most fungi that reacted with the acid media gave conclusive results at the end of 7 days. However, a number produced strong diffusion zones at the end of 1 or 2 days.

RESULTS

REACTION AND GROWTH DATA

The appearance and relation of the brown diffusion zone to the fungus mat, as well as the time required for its development, varied widely with different species. The following system was used to record the reactions of the different species to gallic and tannic acid media:

—, Negative, no brown discoloration of the agar under or about the mat.[6]

+, Diffusion zone light to dark brown, formed under inoculum at center of mat and visible only from under side of dish. In case no growth takes place, a faint brown discoloration under the inoculum.

++, Diffusion zone light to dark brown, formed under most of mat but not extending to margin. Visible from under side only.

+++, Diffusion zone light to dark brown, extending a short distance beyond the margin of the mat and visible from the upper side.

++++, Diffusion zone dark brown, opaque, extending considerably beyond margin of fungus mat.

+++++, Diffusion zone very intense, dark brown, opaque, forming a wide corona about mat. Usually such intense reactions occur with species giving no growth on the medium, and are most common on gallic acid medium.

Usually the brown diffusion zone which formed under the fungus mat would conform to one of the foregoing types. Occasionally a species gave a reaction that did not definitely fall into any of these groups; in such cases special mention is made.

The species of fungi showed interesting growth differences on malt agar containing 0.5 percent of gallic or tannic acid. These differences were caused by the toxicity of the acids. The following system

[6] Certain negative reactors cause decolorization of the medium under the mat. This decolorized zone, which forms most distinctly on tannic acid medium, should not be confused with the brown diffusion zone of typical reactors.

was used to record growth data: 0, no growth; tr., trace, growth confined to inoculum; 10, 15, etc., colony diameters in millimeters. Since growth rates were taken at room temperatures and are variable, they are expressed as a range rather than as an average.

Analysis of growth and reaction data at 7 days showed that the different fungi tested on gallic and tannic acid media could be classified into 10 fairly distinct groups. The behavior group for each individual fungus is given in the last column in table 1. Since this classification is based on growth in relation to type of reaction, no attempt was made to classify those species giving both positive and negative reactions on the same medium. A description of the groups and the number of fungi in each are given on pages 689–693.

TABLE 1.—*Reaction and growth of wood-decaying fungi on gallic and tannic acid media, at the end of 7 days*

Fungus	Type of decay	Isolations	On gallic acid medium		On tannic acid medium		Growth and reaction group No.[1]
			Reaction	Growth	Reaction	Growth	
		Number		*Mm*		*Mm*	
Thelephoraceae:							
Coniophora cerebella Pers.	Brown	5	−	30–50	−	20–35	1
C. suffocata (Peck) Massee	do	2	−	45–60	−	15–20	2
Corticium confluens Fr.	White	2	+++	0	++++	Tr.	4
C. coeruleum (Schrad.) Fr.	do	1	[2]− to +	30	+	Tr.	9
C. galactinum (Fr.) Burt	do	2	+++++	Tr.	+++	20–40	6
C. hydnans (Schw.) Burt		1	+++++	0	+++	10	5
C. investiens (Schw.) Bres.	White	1	+++++	Tr.	+++	20	5
C. lividum Pers.	do	3	++++	25–45	+++	20–30	7
Cytidia salicina (Fr.) Burt		1	+++	0	+++	Tr.	4
Hymenochaete agglutinans Ellis		2	++++	Tr.–30	+++	30–50	8
H. corrugata (Fr.) Lév.	White	1	+++++	15	+++	45	8
H. curisii (Berk.) Morgan	do	1	+++++	20	++++	20	7
H. rubiginosa Dick. ex Lév.	White pocket.	6	++++	Tr.–15	+++	Tr.–10	7
H. tabacina Sow. ex Lév.	White	5	+++++	20–35	++	20–50	7
Peniophora allescheri Bres.	do	1	++++	0	+++	Tr.	4
P. cinerea (Pers.) Cooke	do	2	+++++	Tr.	+++	35	6
P. coccineo-fulva (Schw.) Burt		1	++++	35	++	Tr.	9
P. gigantea (Fr.) Massee		1	[2]+	10	−	0	9
P. incarnata (Pers.) Karst.		2	+++++	Tr.–20	++++	25–40	8
P. nuda (Fr.) Bres.	White	1	+++++	15	+++	35	8
P. pubera (Fr.) Sacc.	do	3	+++++	15–40	+++	20–60	7
Stereum albobadium (Schw.) Fr.	do	1	+++++	Tr.	+++	50	6
S. fasciatum Schw.	do	5	++++	15–40	+++	20–50	7
S. frustulosum (Pers.) Fr.	White pocket.	13	−	25–45	−	20–35	1
S. fuscum Schrad. ex Quél.	White	3	++++	Tr.–10	−	0	9
S. gausapatum Fr.	do	12	++++	10–25	+++	25–35	7
S. murrayi (Berk. and Curt.) Burt	do	6	++++	0	+++	Tr.–10	5
S. pini Fr.	do	2	+++++	Tr.	+++	20–40	6
S. purpureum Pers.	do	4	++++	0–tr.	+++	25–40	6
S. rameale Schw.	do	10	++++	20–50	+++	35–60	7
S. roseo-carneum (Schw.) Fr.	do	1	+++++	Tr.	+++	25	5
S. rugosiusculum Berk. and Curt.	do	1	+++	0	++	40	6
S. sericeum Schw.	do	1	++++	Tr.	+++	25	5
S. subpileatum Berk. and Curt.	White pocket.	10	[3]− to ++	10–15	−	10–20	----------
S. spadiceum Fr.	White	3	++++	Tr.–20	+++	25–40	6
S. sulcatum Burt.	White pocket.	1	+++++	0	+++++	10	5
S. umbrinum Berk. and Curt.	White	1	[2]− to +	35	+++	25	7
Hydnaceae:							
Echinodontium tinctorum Ell. and Ev.	do	5	++++	0	+++	Tr.	4
Hydnum caput-ursi Fr.	do	2	++++	0	++	0	4
H. corolloides Scop.	do	2	++++	0	++	0	4
H. erinaceus Bull.	do	20	++++	0	[4]− to +++	0–tr.	4
H. ochraceum Pers.	do	1	+++	0	++	0	4
H. pulcherrimum Berk. and Curt.		2	−−−−−	65–80	−−−−−	0	3
H. septentrionale Fr.	White	4	++	0	[4]++++	Tr.	4

See footnotes at end of table.

TABLE 1.—*Reaction and growth of wood-decaying fungi on gallic and tannic acid media, at the end of 7 days*—Continued

Fungus	Type of decay	Isolations	On gallic acid medium		On tannic acid medium		Growth and reaction group No.
			Reaction	Growth	Reaction	Growth	
		Number		*Mm*		*Mm*	
Hydnaceae—Continued.							
Irpex cinnamomeus Fr.	White	3	++++	Tr.-10	+++	25-35	6
I. mollis Berk. and Curt.	do	6	+++	0	+++	20-30	6
Phlebia strigosazonata Schw.	do	3	+++++	0	++++	20-30	6
Radulum orbiculare Fr.	do	1	++++	0	+++	Tr.	4
Polyporaceae:							
Daedalea ambigua Berk.	do	3	+++++	0	++++	15-20	5
D. confragosa (Bolt.) Fr.	do	5	++++	0	+++++	10-30	5
D. juniperina Murr.	Brown	5	------	20	------	Tr.	3
D. quercina (L.) Fr.	do	13	−	20-30	−	20-30	1
D. unicolor (Bull.) Fr.	White	3	+++	0	+++	20-35	6
Favolus canadensis Klotz	do	2	++++	0	++++	10	5
Fistulina hepatica (Huds.) Fr.	Dark firm.	15	−	15-20	−	10-25	1
Fomes annosus (Fr.) Cooke	White	5	++++	0-tr.	+++	Tr.-10	5
F. applanatus (Pers.) Gill.	do	22	+++	0-10	++++	10-20	5
F. calkinsii (Murr.) Sacc. and Sacc. D.	do	4	++++	0-tr.	++++	10	5
F. conchatus (Pers.) Gill.	do	9	+++	0-tr.	+++	Tr.	4
F. connatus (Weinm.) Gill.	do	2	++	0	[4]+	0	4
F. densus Lloyd	do	4	+++	Tr.-10	+++	0-tr.	7
F. everhartii (Ell. and Gall.) Schrenk.	do	26	++++	0	++++	10-15	5
F. fomentarius (L.) Gill.	do	12	+++	0	++++	10-20	5
F. fraxineus (Bull.) Cooke	do	2	+++	0	++++	15-20	5
F. fraxinophilus (Peck) Sacc.	do	9	++++	Tr.-10	[4]+++	Tr.-15	7
F. fulvus (Scop.) Gill.	do	1	++++	Tr.	+++	25	5
F. geotropus Cooke	do	2	[2]− to +++	35-40	[2]− to +	10-15	9
F. igniarius (L.) Gill.	do	6	++	0	+++	Tr.	4
F. igniarius var. *laevigatus* (Fr.) Overh.	do	4	+++	0-15	++++	Tr.-20	5
F. igniarius var. *populinus* (Neu.) Camph.	do	6	+++	Tr.-10	+++	Tr.	4
F. lobatus (Schw.) Cooke	do	6	+++	0-10	+++	10-30	5
F. marmoratus Berk. and Curt.	do	2	++++	0	++++	10-15	5
F. meliae (Underw.) Murr.	Brown	2	−	20-45	−	15-30	1
F. officinalis (Vill.) Faull.	do	2	[5]−to+	10	−	0	----------
F. ohiensis (Berk.) Murr.	White	1	+++++	0	++++	0	4
F. pini (Thore) Lloyd.	White pocket.	13	++++	Tr.-15	+++	Tr.-15	7
F. pinicola (Sw.) Cooke	Brown	26	−	15-30	−	10-20	1
F. rimosus Berk.	White	10	++	0-10	++	0-tr.	4
F. robustus Karst.	do	6	++++	Tr.	++++	10-25	5
F. robustus var. *tsugina* (Murr.) Overh.	do	3	+++++	0-tr.	++++	Tr.	4
F. roseus (Alb. and Schw.) Cooke.	Brown	11	[5]−to++	15-25	[5]−to+	Tr.-15	----------
F. scutellatus (Schw.) Cooke	White	4	++++	0	+++	0-15	5
F. subroseus (Weir) Overh.	Brown	3	[5]−to+	25-30	−	10-25	----------
F. tenuis Karst.	White pocket.	3	+++++	0	++++	Tr.-10	5
Sterile *Fomes* on birch	White	13	+++	0	+++	Tr.-15	5
Lenzites betulina (L.) Fr.	do	3	++++	Tr.	+++	30-45	6
L. saepiaria (Wulf.) Fr.	Brown	6	−	10-15	−	Tr.	3
L. striata (Sw.) Fr.	do	1	−	25	−	10	2
L. trabea (Pers.) Fr.	do	4	−	20-30	−	0-tr.	3
Merulius confluens Schw.	White	1	[2]+	20	−	0	9
M. tremellosus (Schrad.) Fr.	do	7	+++++	0-40	++++	20-40	7
Polyporus abietinus (Dicks.) Fr.	White pocket.	1	++++	0	++++	Tr.	4
P. adustus (Willd.) Fr.	White	6	[2]−to++	20-40	+	15-25	7
P. albellus Peck	do	6	+++++	0	++++	Tr.-10	5
P. alboluteus Ell. and Ev.	White[6]	1	+++	55	+++	60	7
P. amygdalinus Berk. and Rav.	Brown	2	−	40	−	40	1
P. anceps Peck	White	10	+++++	0-tr.	[4]++++	Tr.-25	5
P. arcularius (Batsch.) Fr.	----------	1	++++	Tr.	+++	35	6
P. balsameus Peck	Brown	11	[5]−to+	15-25	−	0-tr.	----------
P. berkeleyi Fr.	White	9	++++	0	++++	0-10	4
P. betulinus (Bull.) Fr.	Brown	4	−	35	−	15-20	2
P. borealis Fr.	White	3	++++	0	+++	0-tr.	4
P. brumalis (Pers.) Fr.	do	4	++++	0	++++	20-30	6
P. cinnabarinus (Jacq.) Fr.	do	8	+++++	0-tr.	++++	20-30	6
P. circinatus Fr.	do	5	++++	0-tr.	+	0-tr.	4
P. compactus Overh.	do	11	++++	15-30	+++	20-40	7

See footnotes at end of table.

TABLE 1.—*Reaction and growth of wood-decaying fungi on gallic and tannic acid media, at the end of 7 days*—Continued

Fungus	Type of decay	Isolations	On gallic acid medium		On tannic acid medium		Growth and reaction group No.
			Reaction	Growth	Reaction	Growth	
		Number		*Mm*		*Mm*	
Polyporaceae—Continued.							
P. conchifer (Schw.) Fr.	White	1	++++	0	++++	15	5
P. croceus (Pers.) Fr.	do	17	++++	10–35	++	20–40	7
P. curtisii Berk.	do	3	++++	10–20	+++	15–30	7
P. cuticularis (Bull.) Fr.	do	14	+++	0	++++	Tr.–15	5
P. delectans Peck	do	4	+++	0	+++	Tr.–10	5
P. dichrous Fr.	do	4	−	10	−	0	3
P. distortus (Schw.) Fr.	do	4	++++	10–15	+++	30–40	8
P. dryadeus (Pers.) Fr.	do	2	+++	0	+++	0	4
P. dryophilus Berk.	do	17	++++	10–25	+++	10–25	7
P. fibrillosus Karst.	Brown	1	−	15	−	15	1
P. fissilis Berk. and Curt.	White	12	++++	0	+++	Tr.–10	5
P. fragilis Fr.	Brown	4	−	10	−	0	3
P. frondosus (Dicks.) Fr.	White	10	+++	15–35	+++	10–35	7
P. fumidiceps (Atk.) Sacc. and Trott.	do	1	++++	Tr.	++++	20	5
P. fumosus (Pers.) Fr.	do	2	[2]−to+	15–30	[2]−to+	Tr.–15	7
P. galactinus Berk.	do	7	+++++	0–tr.	++++	Tr.–15	5
P. giganteus (Pers.) Fr.	do	4	+++	0	++++	10	5
P. gilvus (Schw.) Fr.	do	11	+++++	Tr.–15	+++	Tr.–30	5
P. glomeratus Peck	do	10	+++	0	+++	0	4
P. graveolens (Schw.) Fr.	do	8	[4]++	0	[4]+++	Tr.–20	5
P. guttulatus Peck	Brown	2	−	15–25	−	0	3
P. helveolus Rostk.		1	−	40	−	20	2
P. hirsutus (Wulf.) Fr.	White	7	+++++	0	+++	20–40	6
P. hispidus (Bull.) Fr.	do	15	++++	0–10	++++	10–20	5
P. immitus Peck	Brown	3	−	20	−	0	3
P. lucidus (Curt.) Fr.	White	14	++++	0–30	+++	15–40	7
P. ludovicianus (Pat.) Sacc. and Trott.	White pocket.	6	+++	Tr.–10	+++	Tr.	9
P. mutabilis Berk. and Curt.	White	2	+++	0	+	0	4
P. obtusus Berk.	do	11	++++	0	++++	Tr.–15	5
P. oregonensis (Murr.) Kauff.	do	2	++++	0	++++	10	5
P. osseus Kalchbr.		1	−	55	−	0	3
P. palustris Berk. and Curt.	Brown	2	−	15–30	−	15–20	1
P. pargamenus Fr.	White	4	++++	0	++++	Tr.–15	5
P. picipes Fr.	do	1	++	0	+	0	4
P. pubescens (Schum.) Fr.	do	4	+++++	0	+++	30–40	6
P. radiatus (Sow.) Fr.	do	9	+++	Tr.–10	+++	Tr.–15	7
P. resinosus (Schrad.) Fr.	do	9	++++	Tr.–40	+++	10–35	7
P. robiniophilus (Murr.) Lloyd	do	5	+++	0	+++	Tr.–20	5
P. sericeo-mollis Rom.	Brown	7	−	20–35	−	Tr.–10	3
P. schweinitzii Fr.	do	9	−	15–35	−	0	3
P. spraguei B. and C.	do	27	−	30–45	−	20–40	1
P. spumeus (Sow.) Horne	White	3	+++	0–tr.	++++	Tr.–20	5
P. squamosus (Huds.) Fr.	do	3	+++	0	+	0	4
P. subertypus (Murr.) Overh. in comb.	do	1	++++	0	+++	35	6
P. sulphureus (Bull.) Fr.	Brown	23	−	25–45	−	20–45	1
P. supinus (Sw.) Fr.	White	7	+++	0	++++	15–30	5
P. tenuis (Sacc.) Overh.	do	2	++++	0	+++	40–50	6
P. tephroleucus Fr.	Brown	2	[7]−	15	−	0	3
P. tsugae (Murr.) Overh.	White	5	+++	0–tr.	+++	Tr.–25	5
P. tuberaster (Jacq.) Fr.		2	+++	0	+++	0–tr.	4
P. tulipiferus (Schw.) Overh.	White	9	[2]−to++	40–60	[2]−to+	Tr.–30	9
P. umbellatus (Pers.) Fr.		1	+++	0	+	0	4
P. velutinus Fr.	White	1	++++	0	+++	40	6
P. versicolor (L.) Fr.	do	6	+++++	0	+++	30–50	6
P. volvatus Peck	do	3	+++	0	++++	Tr.–10	5
P. vulpinus Fr.	do	1	+++	Tr.	+	0	4
P. zonatis Berk.	White pocket.	5	++	0	[4]+++	Tr.–25	5
Poria andersonii (E. and E.) Neuman.	White	11	++++	0	++++	Tr.–10	5
P. borbonica Pat.	do	1	++++	10	+++	60	8
P. cocos (Schw.) Wolf.	Brown	12	[5]−to+	50–80	−	30–80	---
P. incrassata (Berk. and Curt.) Burt.	do	4	−	20–40	−	0–tr.	3
P. medulla panis (Pers.) Cooke.	White	2	+++++	Tr.–15	+++	30	8
P. mutans Peck	do	1	++++	20	+++	20	7
P. mutans var. tenuis Peck	do	1	++++	20	+++	30	7
P. nigra Berk.	Brown	3	−	25–35	−	25–35	1
P. nigrescens Bres.	White	3	++++	0	++++	Tr.–10	5

See footnotes at end of table.

TABLE 1.—*Reaction and growth of wood-decaying fungi on gallic and tannic acid media, at the end of 7 days*—Continued

Fungus	Type of decay	Isolations	On gallic acid medium		On tannic acid medium		Growth and reaction group No.
			Reaction	Growth	Reaction	Growth	
		Number		*Mm*		*Mm*	
Polyporaceae—Continued.							
P. prunicola (Murr.) Sacc. and Trott.	White	5	++++	Tr.–15	+++	Tr.–20	7
P. pulchella (Schw.) Cooke	do	5	+++++	Tr.–15	+++	15–50	8
P. punctata Fr.	do	6	+++++	0–tr.	++++	10–20	5
P. selecta Karst. ex Rom.	Brown	1	–	45	–	Tr.	3
P. subacida (Peck) Sacc.	White	14	++++	0–10	+++	10–30	8
P. undata (Pers.) Bres.	White pocket	3	++	0	+++	10–20	5
P. vaillantii Fr.	Brown	1	–	25	–	0	3
P. vaporaria Fr.	Brown [6]	2	–	30	–	0–10	2
P. versipora Pers.	White	1	++++	0	++++	15	5
P. weirii (Murr.) Sacc. and Trott.	do	2	+++	20–30	+++	20–30	7
Trametes americana Overh.	Brown	2	–	10–15	–	Tr.	3
T. cubensis (Mont.) Sacc.		1	–	70	–	0	3
T. heteromorpha (Fr.) Lloyd	Brown	1	[5]–to+	20	–	0	----------
T. hispida Bagl.	White	1	++++	0	++++	25	5
T. hydnoides (Sw.) Fr.	do	1	[2]–to++	25	+	10	9
T. malicola B. and C.	Brown	2	–	15	–	Tr.–10	1
T. mollis (Som.) Fr.	White	2	+++	0	++++	Tr.	4
T. rigida Berk. and Mont.	do	1	+++	0	++++	20	5
T. serialis Fr.	Brown	5	[7]–	15–40	–	10–30	1
T. suaveolens Fr.	White	3	++++	0	+++	10–30	5
Agaricaceae:							
Armillaria mellea (Vahl) Quél.	do	12	++++	0–tr.	++++	Tr.–10	5
Clitocybe illudens (Schw.) Sacc.	do	1	++++	10	++++	15	7
C. tabescens (Scop.) Bres.	do	6	++++	0–10	++++	Tr.–10	7
Collybia velutipes (Curt.) Quél.	do	4	+	0	–	0	4
Hypholoma sublateritium Schaeff.	do	6	++++	0–tr.	++++	Tr.–20	5
Lentinus lepideus Fr.	Brown	8	[5]–to+++	Tr.–15	[5]–to+	0–tr.	----------
L. tigrinus (Bull.) Fr.	White	4	++++	Tr.–25	+++	35–45	8
Panus laevis Berk. and Curt.	do	4	+++	0	++	0–tr.	4
P. stipticus (Bull.) Fr.	do	4	++++	0–tr.	++++	Tr.–20	5
Pholiota adiposa (Pers.) Fr.	do	8	+++	0	+++	Tr.–20	5
Pleurotus corticatus Fr.	do	4	+++	0	+++	0–tr.	4
P. ostreatus (Jacq.) Quél.	do	11	+++	0	[4]+++	Tr.–10	5
P. serotinus	do	2	+++	0–20	+++	Tr.–30	7
P. ulmarius (Bull.) Quél.	do	6	–to+	0	–	0	4
Schizophyllum commune Fr.	do	4	–	20–40	[2]++	25–40	10
Ascomycetes:							
Ustulina vulgaris Tul.	do	7	+++++	Tr.–20	+++	20–25	8
Xylaria sp.	do	2	+++++	Tr.	+++	30–40	6
Fungi Imperfecti:							
Strumella coryneoides Sacc. and Wint.	do	6	+++++	20–25	+++	30–50	8

[1] Growth and reaction groups are explained in detail in the text.

[2] These fungi may require from 7 to 14 days to form brown diffusion zones, but the reactions are always positive.

[3] Reaction uncertain; most isolations formed a brown diffusion zone under mat in 7 days. In 14 days zone more intense but certain isolations are consistently negative.

[4] Reaction positive if growth occurs; if no growth, usually positive but occasionally negative.

[5] Reaction usually negative. However, a faint brown diffusion zone may form under center of mat. The same isolation may be negative at one trial but slightly positive at another.

[6] SHOPE, PAUL FRANKLIN. THE POLYPORACEAE OF COLORADO. Ann. Mo. Bot. Gard. 18: 287–456, illus. 1931.

[7] Reaction negative. A widely diffused light-brown indefinite zone may appear around but not under mat. Agar under mat perfectly transparent.

NEGATIVE OR NONREACTING FUNGI

Group 1.—Mat diameter for a given species about equal on both gallic and tannic acid media. 14 fungi. (Fig. 1, *A* and *B*.)

Group 2.—Growth on gallic acid medium. good, mat diameter much greater than on tannic acid medium. Five fungi. (Fig. 1, *C* and *D*.)

Group 3.—Good growth on gallic acid medium, none or only a trace on tannic acid medium. 17 fungi. (Fig. 1, *E* and *F*.)

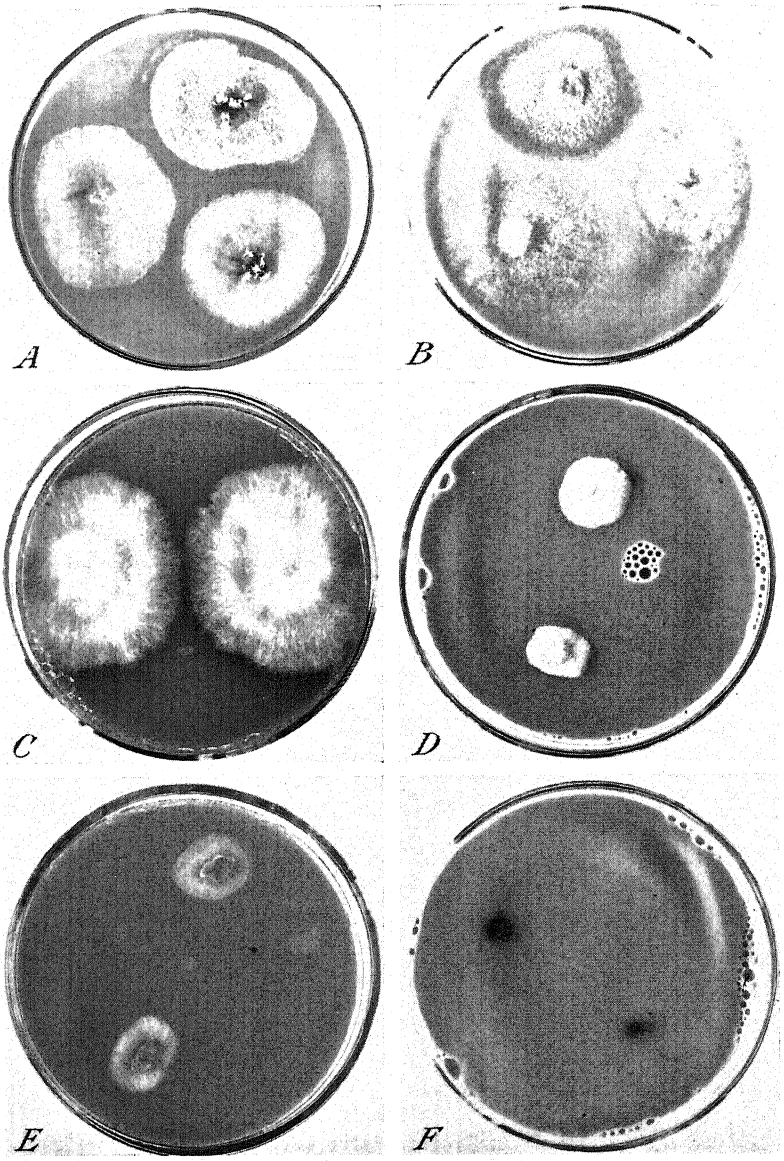


FIGURE 1.—Negative or nonreacting groups. *A* and *B*, Group 1 as illustrated by *Polyporus sulphureus;* mat diameters for a given species approximately the same on gallic (*A*) and tannic (*B*) acid media. *C* and *D*, Group 2, as illustrated by *Coniophora suffocata;* good growth on gallic (*C*) acid medium, poor on tannic (*D*) acid medium. *E* and *F*, Group 3 as illustrated by *Daedalea juniperina;* good growth on gallic (*E*) acid medium, no growth or only a trace on tannic (*F*) acid medium.

POSITIVE-REACTING FUNGI

Group 4.—No growth, or only a trace, on both gallic and tannic acid media. 34 fungi. (Fig. 2, *A* and *B*.)

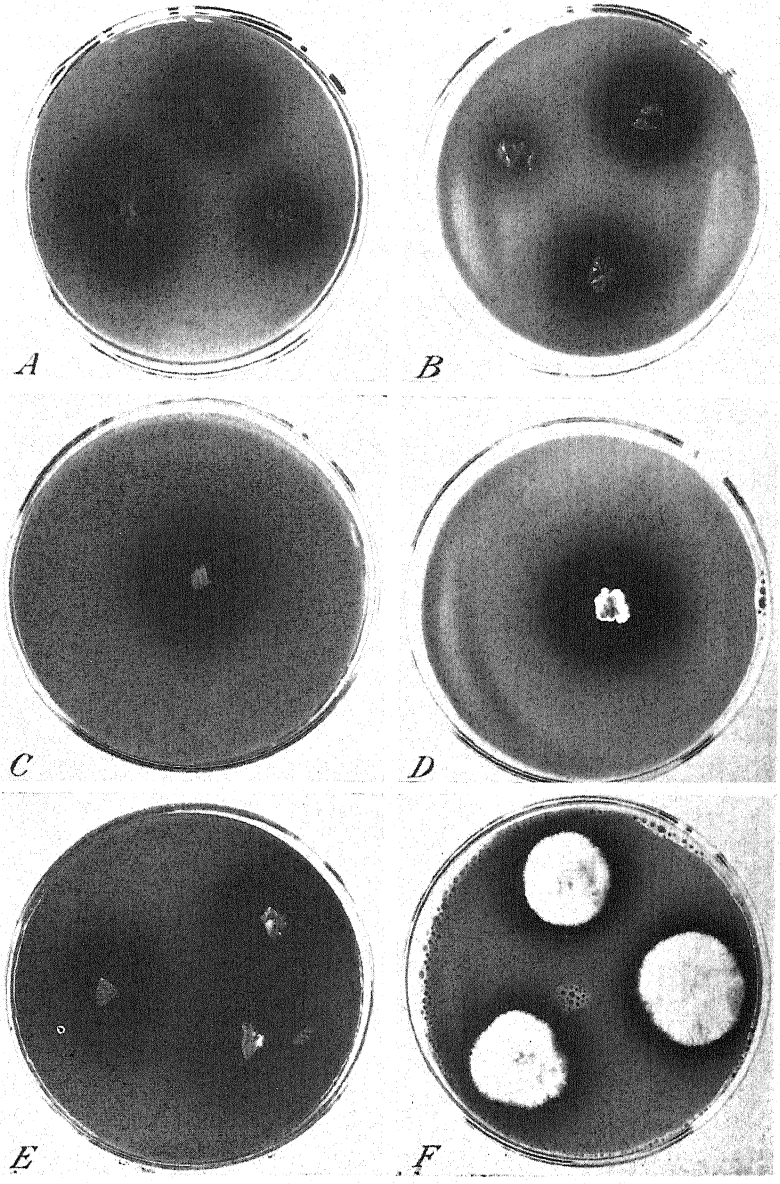


FIGURE 2.—Positive-reacting groups. *A* and *B*, Group 4 as illustrated by *Polyporus glomeratus;* no growth or only a trace on gallic (*A*) and tannic (*B*) acid media. *C* and *D*, Group 5 as illustrated by *Stereum murrayi;* no growth or only a trace on gallic (*C*) acid medium, trace to 25 mm on tannic (*D*) acid medium. *E* and *F*, Group 6 as illustrated by *Irpex mollis;* no growth or only a trace on gallic (*E*) acid medium, growth 25–50 mm on tannic (*F*)

Group 5.—No growth or only a trace on gallic acid medium, mat diameter 25 mm or less on tannic acid. 57 fungi, all strong reactors. (Fig. 2, *C* and *D*.)

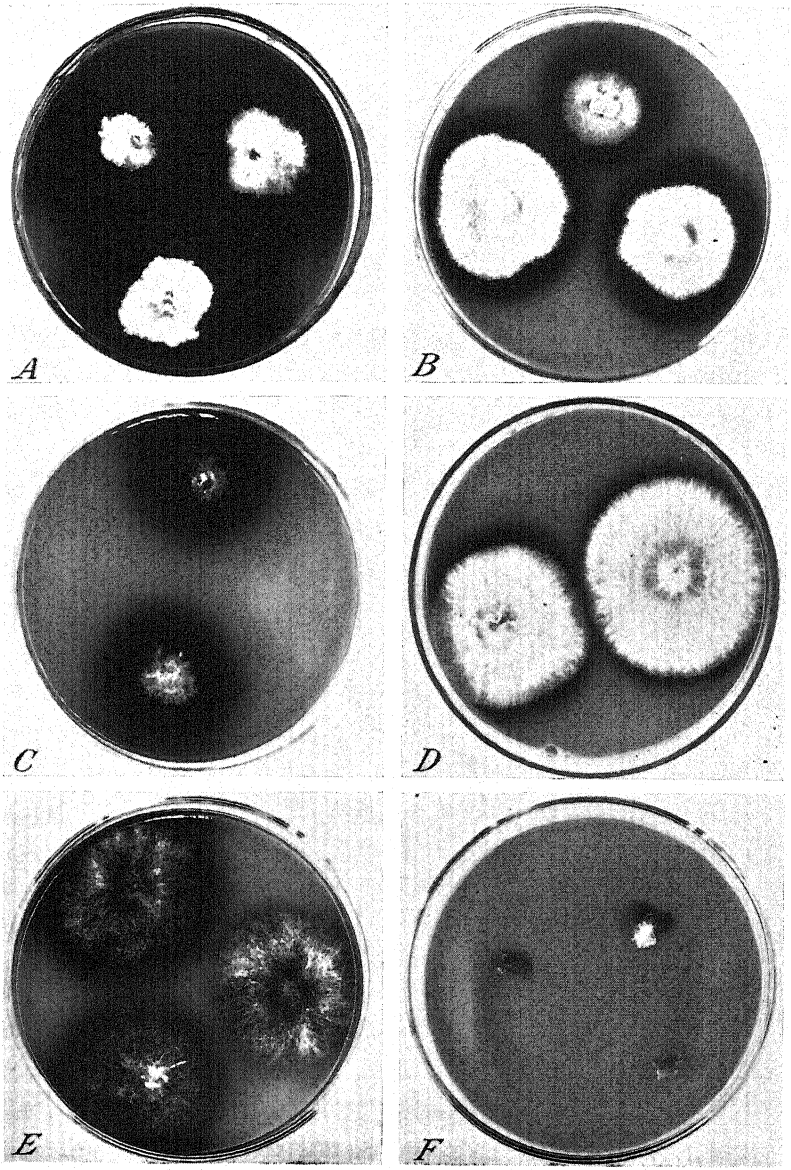


FIGURE 3.—Positive-reacting groups. *A* and *B*, Group 7 as illustrated by *Polyporus compactus;* mat diameters approximately equal on both gallic (*A*) and tannic (*B*) acid media. *C* and *D*, Group 8 as illustrated by *Polyporus distortus;* slight growth on gallis (*C*) acid medium, good growth on tannic (*D*) acid medium. *E* and *F*, Group 9 as illustrated by *Polyporus tulipiferus;* good growth on gallic (*E*) acid medium, none to a trace on tannic (*F*) acid medium.

Group 6.—Growth none or only a trace on gallic acid medium, growth 25–50 mm on tannic acid. 22 fungi. (Fig. 2, *E* and *F*.)

Group 7.—Mat diameters about equal on both media. 31 fungi. (Fig. 3, *A* and *B*.)

Group 8.—Fair growth on gallic acid medium, good growth on tannic acid medium. 12 fungi. (Fig. 3, *C* and *D*.)

Group 9.—Good growth on gallic acid medium, none to trace on tannic acid medium, often weak reactors, some requiring 14 days for definite results. Nine fungi. (Fig. 3, *E* and *F*.)

FUNGI HAVING NEGATIVE OR POSITIVE REACTION, DEPENDING ON MEDIUM

Group 10.—Reaction negative on gallic acid medium, positive on tannic acid medium, with good growth on both. One fungus (*Schizophyllum commune*).

DISCUSSION

Naturally, since arbitrary limits have been prescribed for the above-mentioned groups, certain fungi should be expected to show growth behavior which may fall within two or more groups. Each fungus is placed in a group on the basis of its most consistent and characteristic growth behavior, and the relative consistency with which different isolates fall into a given group may vary for the different species. For this reason, in comparing growth groups of two different species, more significance should be attached to growth reactions involving widely separated groups such as groups 4 and 9 than to those involving closely related groups such as 4 and 5 or 5 and 6. This applies to both positive and negative reactors. In order to demonstrate the consistency with which a given fungus would fall into a definite group on repeated trials of the same isolate, or with a number of isolates, the data for a few fungi, for which several isolates were available, have been analyzed. Ninety-five percent of the recorded conclusive trials place *Polyporus compactus* in group 7 and 5 percent in group 6 (21 trials); 54 percent place *P. cuticularis* in group 5 and 46 percent in group 4 (22 trials); 73 percent place *P. dryophilus* in group 7, 21 percent in group 9, and 5 percent in group 4 (19 trials); 82 percent place *P. hispidus* in group 5 and 17 percent in group 4 (17 trials); 97 percent place *P. spraguei* in group 1 and 3 percent in group 3 (33 trials); 73 percent place *P. supinus* in group 5 and 27 percent in group 6 (15 trials); and 72 percent place *Poria andersonii* in group 5 and 28 percent in group 4 (18 trials).

The foregoing growth and reaction groups are constant and distinct enough to be useful in the identification of certain fungi. For example, cultures of *Polyporus pargamenus* and *P. tulipiferus* are sufficiently similar to be confused in the laboratory. On 0.5-percent gallic and tannic acid media, however, the reaction of the former falls under group 5, that of the latter under group 9. The differences between the two species on these media cannot be confused. In many cases identification of isolations from unnamed sporophores may be assured if the type of decay associated with the sporophore can be determined. The oxidase test is useful in such cases as well as in the general determination of decay types for fungi taken from incipient decay before the typical stage manifests itself. *P. schweinitzii* and *P. circinatus* sporophores are similar, but cultures of the two fungi can be readily separated on gallic and tannic acid media, as the former is a nonreactor while the latter is a positive reactor.

A number of other instances where the oxidase test has been of value could be cited, but the few listed here give a general idea of cases in which the oxidase reaction, correlated with other information, has been of particular value.

A column showing type of decay is included in table 1 to give some idea of the kind of rot that is usually associated with each fungus. The type of rot has not been determined by pure-culture methods but by the association of sporophores with the rots in question. In many cases, as in certain groups of the Thelephoraceae, where no definite association of rot and fungus has been found, the space is left blank.

As used in the paper, the term "brown rot" refers only to the brown cubical type of decay. Most of the others are classified as white rots, although many are somewhat colored owing to the color of the wood or to changes associated with incipient stages of infection. For instance, *Stereum frustulosum*, *S. subpileatum*, and *Hydnum erinaceus* are usually present in brown-stained wood, but the wood is white or light-colored in the final stages of decay.

Of the 210 fungi that were tested for the presence of oxidases 36 were negative, i. e., they did not form brown diffusion zones on either medium; 8 were inconsistent, i. e., they were usually negative, but certain isolations gave slight brown diffusion zones on one or both media; 165 were positive; and 1 (*Schizophyllum commune*) gave consistently negative results with gallic acid medium but positive results with tannic acid, with good growth on both.

Of the 36 fungi that were consistently negative, 29 are associated with brown carbonizing rots;[7] the type of decay for 4 fungi could not be checked. *Fistulina hepatica* causes a darkening of the wood but not a typical brown rot; *Stereum frustulosum* causes a white pocket rot, which produces dark discoloring of the wood about the decayed pockets; and *Polyporus dichrous* is associated with what appears to be a definite white rot. Of the eight fungi giving inconsistent, but mainly negative results, seven are definitely brown rot fungi while *S. subpileatum* is associated with a white pocket rot with darkening of the surrounding wood.

Of the 165 fungi giving consistently positive oxidase reactions, 156 are associated with white rot;[8] the type of decay was unknown for 9 species. *Schizophyllum commune*, associated with a white decay, was negative on gallic acid but positive on tannic acid medium, with good growth on both.

Eighty percent of the negative reactors were correctly diagnosed as brown rot fungi and 96 percent of the positive reactors were cor-

[7] The majority of brown rot fungi showed no signs of reaction on either gallic or tannic acid medium. This was especially true of those species producing white mycelia in culture. The species that gave inconsistent results usually formed yellow, buff, or brown mycelia in culture, and it was often difficult to decide whether the faint discoloration, as seen from the under side of the Petri dish, was due to the color of the mat or to reaction with the medium. This was especially true on the transparent gallic acid medium.

[8] White rot fungi showed considerable variation in the intensity of the brown diffusion zone and in the time required for its formation. Very strong reactors gave a definite zone in 1 or 2 days. The great majority produced definite results in 7 days while only a few fungi required a longer time for the zone to form. Strongest reactors gave positive results with little or no growth, especially on gallic acid. A few gave results only with growing mycelium. For this reason tests showing no reaction and no growth on either medium were considered inconclusive, but tests showing a reaction without growth were considered conclusive. Since all fungi do not react equally well with both tannic and gallic acid media both should be used when the purpose of the test is to determine decay type by the oxidase reaction.

rectly diagnosed as white rot fungi. Thus Bavendamm's generalization, that brown rot fungi are negative when tested for oxidases by the use of gallic and tannic acids and that white rot fungi give positive reactions with the same media is essentially correct.

SUMMARY

Two hundred and ten fungi were tested for oxidases, using Bavendamm's gallic and tannic acid method.

Twenty-nine, or 80 percent, of the fungi associated with brown carbonizing rots gave no reaction with gallic or tannic acid media. One (*Fistulina hepatica*), associated with a dark firm rot, was negative, as was *Stereum frustulosum*, associated with a white pocket rot, and *Polyporus dichrous*, associated with a white rot. Four whose rots were unknown gave no reaction. Seven fungi associated with brown carbonizing rots gave inconsistent reactions.

One hundred and fifty-six, or 96 percent, of those fungi associated with white rots gave positive reactions. Nine specimens whose rots were unknown also gave positive reactions. *Stereum subpileatum*, associated with a white pocket rot similar to *S. frustulosum*, gave mostly positive results on gallic acid but was negative on tannic acid. *Schizophyllum commune*, a white rot fungus, was negative on gallic acid medium but positive on tannic acid medium, with good growth on both media.

With white rot fungi the intensity of reaction and the time required for the reaction to take place showed considerable variation.

Growth behavior for different species on gallic acid and tannic acid media showed variations of value in the cultural identification of fungi. Ten different growth and reaction groups are described and the species are listed under these groups.

AN ANALYSIS OF THE BLOOD OF THE SIXTH-INSTAR SOUTHERN ARMYWORM (PRODENIA ERIDANIA) [1]

By FRANK H. BABERS
Associate biochemist, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture

INTRODUCTION

The body cavity of insects contains only one tissue fluid, the so-called blood, or hemolymph. The composition of this blood varies greatly from species to species, and there is also marked variation in individuals of the same species. It becomes important, therefore, to determine the approximate concentration of the various constituents in the blood of insects that are being used in experiments on insect physiology. Mature (sixth-instar) larvae of the southern armyworm (*Prodenia eridania* (Cram.)) have been used in large numbers in these laboratories for such experiments. In order that a perfusion fluid similar to the normal blood might be prepared, a quantitative analysis of the blood of this insect was made. It was also necessary to know the approximate normal concentrations of the various constituents of the blood before attempting a study of the effect of feeding various foodstuffs and certain toxic substances on the processes of digestion and absorption. The results of the several analyses are recorded in this report.

Most of the analytical procedures were developed for use with human blood, and, with few exceptions, they were found entirely satisfactory for use with insect blood. Many of the methods are recorded by Peters and Van Slyke (*28*),[2] and the modifications of the original procedures that these authors recommend have been followed.

EXPERIMENTAL DATA

GENERAL REACTIONS

The blood from mature larvae of the southern armyworm is a viscous fluid ranging in color from bright green to a dirty, nondescript yellow. It begins to clot almost immediately after being drawn from the insect, the clotting apparently being of the type classified by Yeager and Knight (*37*) as cell coagulation.

The blood from the southern armyworm does not cause clotting when added to solutions of fibrinogen or thrombin obtained from normal horse serum.

The volume of blood varies considerably among individuals and seems to be dependent on both the age of the larvae and the type of food on which it has fed. The quantity of blood obtained from one insect was usually from 0.07 to 0.2 ml, with an average of 0.12 ml.

[1] Received for publication May 27, 1938; issued November 1938.
[2] Italic numbers in parentheses refer to Literature Cited, p. 704.

On exposure to air the blood darkens rapidly from the surface down, and is soon almost black. This darkening is probably due to the presence of tyrosinase, since a suspension of tyrosine turns black in a few hours after a small quantity of insect blood has been added.

Both clotting and darkening are prevented and the insect is killed by immersion in water at 60° C. for 1 minute. There is no visible coagulation of plasma[3] proteins from this treatment.

The Millon, Hopkins-Cole, xanthoproteic, Adamkiewicz, biuret, and Molisch tests were all strongly positive, as was the sulphuric acid-petroleum ether test for carotinoids. The Pettenkofer test for bile salts was also positive. The ether extract of a sample of blood boiled with an equal volume of 12 N hydrochloric acid and a pinch of naphthoresorcin (Tollens' reagents) was a bright reddish purple. This color was entirely different from the color given by glucuronic or galacturonic acid. The sodium nitroprusside and bromine tests were negative.

DIGESTIVE ENZYMES IN THE BLOOD

Invertase and lipase were demonstrated in whole blood by using 1 percent of sucrose and cream as the respective substrates. Amylase was not found, nor were the proteolytic enzymes pepsin, trypsin, and erepsin. In man proteolytic enzymes, together with their antibodies, are found in serum. These antibodies render the enzymes inactive and prevent digestion of the blood proteins. Extraction of the blood with toluene, chloroform, or certain other organic solvents either removes these antienzymes or renders them inactive. Even after extraction with toluene and chloroform, no proteolytic enzymes were demonstrated in the blood of the southern armyworm.

HYDROGEN-ION CONCENTRATION

The pH value of the blood was determined electrometrically with the micro quinhydrone electrode described by Cullen (*15*). Blood was obtained by snipping off a leg while holding the insect under mineral oil. At 24.5° C. the pH value of the blood from six larvae varied between 6.40 and 6.67, with an average of 6.53.

OSMOTIC PRESSURE

The osmotic pressure of whole freshly drawn blood was determined by the freezing-point method, with use of a copper-constantan thermocouple. Δ was found to be $-0.84°$ C. This corresponds to an osmotic pressure of 10.12 atmospheres, based on a molecular freezing-point lowering of 1.86° and an osmotic pressure of 22.4 atmospheres.

BODY TEMPERATURE

Incidental to the determination of the freezing point of the blood, the body temperature of the insect was determined by inserting a copper-constantan thermocouple in the anus of insects wired down on cork. When the room temperature was 23.8° C., the average temperature of six insects after equilibrium was reached was 22.3°, or 1.5° below room temperature.

[3] In all cases where the term "plasma" is used, reference is to the fluid obtained after centrifugation of blood from such heat-treated insects, whereas the term "serum" refers to the fluid obtained by centrifugation of blood that clotted after being drawn from living insects.

DENSITY

Blood from larvae killed by heating at 60° C. for 45 seconds was weighed in a micropycnometer at 25° and the weight compared with that of the same volume of distilled water at 25°. The density was 1.032 g per cubic centimeter.

OXYGEN CAPACITY

Blood from live larvae was collected under mineral oil to retard the darkening that begins soon after the blood is drawn from the insect and exposed to air. Blood so drawn remained undarkened for at least 2 hours. After standing for 15 minutes in the ice box, the tube was centrifuged and the clear serum pipetted off. One-milliliter samples were analyzed for oxygen capacity in the Van Slyke manometric apparatus by both the regular Van Slyke method and the following modification: The sample was washed into the gas chamber with 2.5 ml of 0.9-percent sodium chloride solution and equilibrated with air by shaking for 2 minutes. A drop of caprylic alcohol was added, followed by 0.2 ml of 5-percent sodium cyanide solution, and the dissolved gases were released by evacuating for 2 minutes. The carbon dioxide was absorbed with 1 ml of normal sodium hydroxide solution and the manometer read. One milliliter of alkaline pyrogallic solution (1 volume of 22-percent pyrogallic acid plus 4 volumes of 10-percent potassium hydroxide) was then added and the manometer again read after 2 minutes had been allowed for drainage and absorption. The difference in the two readings multiplied by Van Slyke's factors gives the volume-percent of oxygen gas in the sample.

Determinations were also made with whole blood. It was difficult to obtain homogeneous samples of whole blood because of the rapid clotting, but the clot was broken up as much as possible before the sample was taken. Clotting was so retarded that homogeneous samples were obtained when the blood was allowed to drop directly from the insect into an equal volume of saturated sodium oxalate solution. Blanks in which a physiological salt solution was substituted for the insect blood were run with each determination. In every experiment the oxygen capacity of the insect blood and serum was slightly less than that of normal salt solution.

As a check on the methods of analysis, the oxygen capacity of the serum from lobster (*Homarus americanus*) was determined. This blood is known to contain the oxygen carrier hemocyanin in appreciable quantities.

The results of these determinations are shown in table 1.

TABLE 1.—*Oxygen content of the blood of the southern armyworm*

Manner of liberating gases	Material tested	Temperature	Oxygen capacity		Oxygen unaccounted for by blank
			Material	Blank	
		°C.	*Volume-percent*	*Volume-percent*	*Volume-percent*
Van Slyke technique	Blood-saturated sodium oxalate (1:1)	24	2.39	2.48	----------
	Whole blood	25	2.45	2.53	----------
	Serum	26	1.88	2.02	----------
Sodium cyanide	Whole blood	15	1.64	1.73	----------
	Serum	24	1.81	1.98	----------
	Lobster blood	21	2.96	2.03	0.93

CHEMICAL ANALYSES

Nonprotein nitrogen was determined on the filtrate obtained by treating plasma with nitrogen-free trichloroacetic acid and centrifuging. The micro-Kjeldahl method, with potassium persulphate and copper sulphate as catalysts, was used. The total plasma nitrogen was determined by micro-Kjeldahl analysis. The protein content was obtained by subtracting the amount of nitrogen in the protein-free filtrate and multiplying the difference by the factor 6.25. The general nature of these proteins has not yet been determined.

The micro-Van Slyke method was used for determining amino nitrogen in the protein-free filtrate obtained on treating plasma with sodium tungstate reagent.

The apparent "creatinine" in the blood was determined by the method of Folin and Wu (*18*).

The blood was analyzed for urea by the xanthydrol colorimetric method of Beattie (*2*).

The uric acid in the Folin and Wu protein-free filtrate of serum was determined colorimetrically by Benedict's direct method (*3*, *4*).

The colorimetric method of Shohl and Bennett (*29*) was used for the determination of potassium. Attempts to use cobaltinitrite methods were unsuccessful, as checks could not be obtained.

Blood was analyzed for sodium by the Butler and Tuthill (*12*) modification of the method of Barber and Kolthoff.

Calcium was determined by the modified Halverson and Bergeim method described by Peters and Van Slyke (*28*, *p. 767*).

Magnesium in the filtrate from the calcium determination described above was precipitated as magnesium ammonium phosphate (*11*), and the phosphate was determined by Tisdall's colorimetric method (*32*).

A measured volume of pooled blood from a number of insects that had been killed and their blood fixed by immersion in water at 60° C. for 1 minute was added to 10 volumes of 10-percent trichloroacetic acid solution in a 15-cc centrifuge tube. After standing for 10 minutes, the tubes were centrifuged and the supernatant liquid was poured quantitatively into a 100-ml Kjeldahl flask. The precipitate was dissolved in a small volume of normal sodium hydroxide solution and rinsed into another Kjeldahl flask. Both solutions were boiled down to small volume, ashed, and the copper sulphide was precipitated by the method of Ansbacher, Remington, and Culp (*1*). The copper sulphide was dissolved in concentrated nitric acid, the acid removed by evaporation on the steam bath, and the copper determined colorimetrically by the method of Callan and Henderson (*13*) with the use of sodium diethyl dithiocarbamate.

Inorganic sulphur in the blood was determined as barium sulphate by the gravimetric procedure of Loeb and Benedict (*21*). When 15 ml of the protein-free filtrate was hydrolyzed with 4 ml of normal hydrochloric acid for 20 minutes, the barium sulphate precipitated gave inorganic plus ethereal sulphur. Nonprotein sulphur was determined on 10 ml of the protein-free filtrate after hydrolysis with the zinc nitrate oxidizing mixture described by Denis and Reed (*16*).

Chlorides were determined on 0.2-ml samples of whole blood by Patterson's method (*27*), after digestion by the open Carius method. There were 119.8 mg of chlorine per 100 ml of blood, which corresponds to 197.5 mg of sodium chloride.

Total reducing substances were found by the Somogyi micro modification of the Shaffer-Hartmann method (*30*). This method proved more satisfactory than the Hagedorn-Jensen procedure. Fermentable substances were determined by treatment with yeast according to the procedure described by Peters and Van Slyke (*28, p. 479*).

The colorimetric method of Mendel and Goldscheider (*25*) was used to determine lactic acid. Only a trace was found by this procedure.

Pooled blood from insects killed by immersion in water at 60° C. for 1 minute was analyzed for glycogen by the method of Creveld (*14*), the only modification being that the glucose liberated was determined with the micro Shaffer-Hartmann-Somogyi reagent I (*30*). Glycogen was obtained from the glucose value by multiplying by the factor 0.927.

Total lipoids (lipids), fatty acids, and cholesterol were determined by the chromate oxidation method as described by Bloor (*7*). The phospholipoids were isolated and determined according to Bloor's procedure (*8*). The direct extraction and colorimetric method of Leiboff (*20*) and the method of Bloor, Pelken, and Allen (*9*) were used in the determination of cholesterol. Samples corresponding to 2 ml of blood were used in each case. Some trouble was experienced with the colorimetric method because of the formation of a yellowish color which was extremely difficult to compare with the standard. This was overcome by washing the chloroform extract with water and drying with calcium chloride before developing the color. The use of a red filter in the colorimeter was also of benefit.

The total phosphorus in whole blood was determined by Tisdall's (*32*) colorimetric method after digestion with a mixture of nitric and sulphuric acids. Inorganic phosphorus was determined by the same method after removal of the proteins with trichloroacetic acid.

The amounts of the various substances found in 100 ml of blood are as follows:

	Milligrams
Nonprotein nitrogen	401. 4
Total nitrogen	568. 4
Amino nitrogen	235. 2
Proteins (protein nitrogen×6.25)	1, 043. 8
"Creatinine" (apparent)	8. 0
Urea	6. 2
Uric acid	14. 8
Potassium	155. 0
Sodium	51. 2
Calcium	36. 75
Magnesium	17. 2
Copper, total	4. 93
Copper, in protein-free filtrate	2. 94
Copper, in protein precipitate	1. 99
Sulphur, inorganic	30. 7
Sulphur, ethereal	0. 4
Sulphur, total nonprotein	44. 4
Chlorine	119. 8
Chlorine (as sodium chloride)	197. 5
Total reducing substances (as glucose)	65. 9
Reducing substances fermented by yeast (as glucose)	11. 1
Reducing substances not fermented by yeast (as glucose)	54. 8
Lactic acid	Trace
Glycogen	3. 29
Lipoids, total	320. 5
Phospholipoids	99. 0
Cholesterol	12. 8
Phosphorus, total	123. 3
Phosphorus, inorganic	17. 59

DISCUSSION

In a paper that has been widely quoted, Muttkowski (*26*) reported the presence of copper and oxygen in the blood of the various insects he tested, but he gave no quantitative results. He found also that the bloods gave positive tests with benzidine, *o*-tolidine, and guaiac. He failed to find copper in human blood. From these experiments he drew the sweeping conclusions that all insect bloods contain a respiratory protein, and that this respiratory protein is hemocyanin, the copper-containing oxygen carrier found in the blood of many marine invertebrates.

The almost universal occurrence of copper is well established. Its presence in plants, human blood and tissue, and the blood of many other animals has been demonstrated by McHargue (*22*, *23*), Warburg (*34*), Elvehjem, Steenbock, and Hart (*17*), and others, and so its mere presence in insect blood would seem to be without special significance. Melvin (*24*) has shown that certain insects accumulate copper, and that the cast skins of others have more copper than the insects producing them. The fact that copper was found in such relatively large quantities by Melvin and also by the author in the insects studied may very well mean that copper has a definite physiological function in insects, but at the present time there seems to be no real evidence for stating just what this function may be.

The qualitative presence of oxygen in insect blood as reported by Muttkowski is, of course, valueless as evidence either for or against the presence of a respiratory protein, since there is no qualitative method of distinguishing between dissolved oxygen and oxygen bound by any of the known respiratory proteins. Oxygen capacities for insects and other invertebrates of approximately the same order of magnitude as those for mammals were reported by Griffiths (*19*). Much of his work, however, has been refuted by Winterstein (*36*), and in the light of later evidence his values must be considered far too high.

Bishop (*5*) found no evidence of a respiratory protein and found also that the quantity of oxygen present in the blood of the honeybee larva was not appreciably more than would be physically dissolved. Bishop used the regular Van Slyke apparatus and technique.

In the present work the writer has obtained similar results with the blood of the southern armyworm larva. It is well known that oxyhemocyanin is not reduced by ferricyanide as is hemoglobin. Oxyhemocyanin is reduced by cyanides, however, and this fact has been utilized by many investigators in determining the oxygen capacity of bloods known to contain hemocyanin. For this reason, although there is no evidence that insect blood contains a hemocyanin, a sodium cyanide solution, as well as the regular Van Slyke reagent, was used during the course of the analyses. The oxygen obtained from southern armyworm blood was in all cases slightly less than was obtained from equal volumes of physiological saline.

In tests with benzidine, *o*-tolidine, and guaiac, the writer obtained positive results only with benzidine, and then only when a saturated solution was used. Little or no significance can be attached to these tests, since positive reactions are given by raw milk, pus, saliva, many plant extracts, potatoes, and other substances in addition to the respiratory proteins. Diastases, as well as the so-called oxidation enzymes

so common in plant fungi, seeds, and bacteria, also give positive reactions. Ferric and cupric salts react positively, as do many other inorganic materials.

These experiments seem to indicate that, if hemocyanin or any other respiratory protein is present in southern armyworm blood, it occurs in such small quantities as to play no part in the normal respiration of the insect.

Although Muttkowski concluded that hemocyanin is present in insect blood, he states: "No doubt some of this reaction [the test for oxygen in the blood] was due to oxygen dissolved in the blood serum, although the serum has no power to combine with oxygen like a respiratory protein." This conclusion, if supported by experimental evidence, should have prevented him from suggesting the presence of hemocyanin in insect blood, since serum is the only portion of blood from which a hemocyanin has been isolated. As far as the author knows, it has never been demonstrated in cells.

The writer's value for the oxygen capacity of lobster blood is similar to that reported by Stedman and Stedman (*31*) in their work on hemocyanins from various sources.

At the present time no explanation is made for any seeming abnormalities in the composition of the blood. The ratio of amino nitrogen to nonprotein nitrogen is extremely high in comparison with this ratio in vertebrates, but the total nitrogen and protein nitrogen seem low. The urea and uric acid content of the blood is high, but this is to be expected, since it has been shown that insects excrete most of their waste nitrogen in the form of uric acid (*35*).

Magnesium is present in high concentration, but this also seems to be generally true with insect blood. Inorganic sulphur is high, but reducing substances, especially substances fermentable by yeast, are extremely low. This probably accounts for the fact that only a trace of lactic acid was found. The composition of the nonfermentable portion is not known, but the reduction is probably due in part to uric acid. The high concentration of glycogen in the blood may also explain the low glucose content to some extent.

Voit (*33*) has shown that glycogen can be formed in mammals from various monosaccharides and disaccharides. Of the substances from which glycogen is usually formed, carbohydrates are probably the most common in the normal diet of the southern armyworm. The insect stores large quantities of glycogen in its fat body for use during pupation. It is probable, therefore, that the insect possesses an efficient glycogen-manufacturing mechanism by which carbohydrates are transformed into glycogen. This in turn may account for the relatively small quantities of free reducing sugars normally present in the blood.

Both total phosphorus and inorganic phosphorus are very high as compared with the phosphorus in human serum, but the values are not unusual for insects. Brecher (*10*) found phosphorus to be high in *Pieris brassicae;* and Bishop, Briggs, and Ronzoni (*6*) found a similar condition in the blood of honeybee larvae.

Cholesterol is such an important constituent of human blood that the relatively small quantities found in the larval blood seem especially interesting.

SUMMARY

The blood (hemolymph) of sixth-instar larvae of the southern armyworm (*Prodenia eridania* (Cram.)) has been analyzed.

A brief description has been given of the blood with some of its physical properties, viz, approximate blood volume, color, density, osmotic pressure, and pH value.

The blood gives positive Millon, Hopkins-Cole, xanthoproteic, Adamkiewicz, biuret, Molisch, Pettenkofer, and carotinoid reactions. The sodium nitroprusside and bromine tests were negative.

Some of the digestive enzymes of the blood have been determined. Invertase and lipase were demonstrated, but amylase, pepsin, trypsin, and erepsin were not found.

The body temperature of the insect was found to be 1.5° C. below room temperature.

The oxygen capacity was found to be slightly less than that of normal saline, and so it was concluded that no respiratory protein or pigment was present.

Total nitrogen, nonprotein nitrogen, amino nitrogen, proteins, apparent "creatinine," urea, uric acid, potassium, sodium, calcium, magnesium, copper, total, inorganic, and ethereal sulphur, chlorides, total reducing substances, substances fermented by yeast, glycogen, total lipoids, phospholipoids, cholesterol, and total and inorganic phosphorus have been determined quantitatively.

No evidence was obtained to account for the seeming abnormal concentrations of several of the constituents.

LITERATURE CITED

(1) Ansbacher, Stefan, Remington, Roe E., and Culp, F. Bartow.
1931. copper determination in organic matter. Indus. and Engin. Chem., Analyt. Ed. 3: 314–317.

(2) Beattie, Florence.
1928. a micro-method for the colorimetric determination of urea in blood. Biochem. Jour. 22: 711–712.

(3) Benedict, Stanley R.
1922. the determination of uric acid in blood. Jour. Biol. Chem. 51: 187–207.

(4) ———
1922. the determination of uric acid. Jour. Biol. Chem. 54: 233–238.

(5) Bishop, George H.
1923. body fluid of the honey bee larva. I. osmotic pressure, specific gravity, pH, O_2 capacity, CO_2 capacity, and buffer value, and their changes with larval activity and metamorphosis. Jour. Biol. Chem. 58: 543–565, illus.

(6) Bishop, G. H., Briggs, A. P., and Ronzoni, E.
1925. body fluids of the honey bee larva. II. chemical constituents of the blood, and their osmotic effects. Jour. Biol. Chem. 66: 77–88.

(7) Bloor, W. R.
1928. the determination of small amounts of lipid in blood plasma. Jour. Biol. Chem. 77: 53–73.

(8) ———
1929. the oxidative determination of phospholipid (lecithin and cephalin) in blood and tissues. Jour. Biol. Chem. 82: 273–286.

(9) ——— Pelken, K. F., and Allen, D. M.
1922. determination of fatty acids (and cholesterol) in small amounts of blood plasma. Jour. Biol. Chem. 52: 191–205, illus.

(10) BRECHER, LEONORE.
1929. DIE ANORGANISCHEN BESTANDTEILE DES SCHMETTERLINGSPUPPENBLUTES (SPHYNX PINASTRI, PIERIS BRASSICAE) VERÄNDERUNGEN IM GEHALT AN ANORGANISCHEN BESTANDTEILEN BEI DER VERPUPPUNG (PIERIS BRASSICAE). Biochem. Ztschr. 211: [40]–64.
(11) BRIGGS, A. P.
1924. SOME APPLICATIONS OF THE COLORIMETRIC PHOSPHATE METHOD. Jour. Biol. Chem. 59: 255–264.
(12) BUTLER, A. M., and TUTHILL, ELIZABETH.
1931. AN APPLICATION OF THE URANYL ZINC ACETATE METHOD FOR DETERMINATION OF SODIUM IN BIOLOGICAL MATERIAL. Jour. Biol. Chem. 93: 171–180.
(13) CALLAN, THOMAS, and HENDERSON, J. A. RUSSELL.
1929. A NEW REAGENT FOR THE COLORIMETRIC DETERMINATION OF MINUTE AMOUNTS OF COPPER. Analyst 54: 650–653.
(14) CREVELD, S. VAN.
1934. INVESTIGATIONS ON GLYCOGEN DISEASE. Arch. Disease Childhood 9: 9–26.
(15) CULLEN, GLENN E.
1929. AN IMPROVED FORM OF THE QUINHYDRONE ELECTRODE. Jour. Biol. Chem. 83: 535–538, illus.
(16) DENIS, W., and REED, LUCILLE.
1926. METHODS FOR THE DETERMINATION OF SOME OF THE NON-PROTEIN SULFUR COMPOUNDS OF BLOOD. Jour. Biol. Chem. 71: 191–204.
(17) ELVEHJEM, C. A., STEENBOCK, H., and HART, E. B.
1929. IS COPPER A CONSTITUENT OF THE HEMOGLOBIN MOLECULE? THE DISTRIBUTION OF COPPER IN BLOOD. Jour. Biol. Chem. 83: 21–25.
(18) FOLIN, OTTO, and WU, HSIEN.
1919. A SYSTEM OF BLOOD ANALYSIS. Jour. Biol. Chem. 38: 81–110, illus.
(19) GRIFFITHS, A. B.
1893. ON THE BLOOD OF THE INVERTEBRATA. Roy. Soc. Edin. Proc. (1891–92) 19: 116–130.
(20) LEIBOFF, S. L.
1924. A SIMPLIFIED METHOD FOR CHOLESTEROL DETERMINATION IN BLOOD. Jour. Biol. Chem. 61: 177–180, illus.
(21) LOEB, ROBERT F., and BENEDICT, ETHEL M.
1927. INORGANIC SULPHATES IN HUMAN BLOOD. Jour. Clin. Invest. 4: 33–36.
(22) MCHARGUE, J. S.
1925. THE OCCURRENCE OF COPPER, MANGANESE, ZINC, NICKEL, AND COBALT IN SOILS, PLANTS, AND ANIMALS, AND THEIR POSSIBLE FUNCTION AS VITAL FACTORS. Jour. Agr. Research 30: 193–196.
(23) ———
1925. THE ASSOCIATION OF COPPER WITH SUBSTANCES CONTAINING THE FAT-SOLUBLE A VITAMIN. Amer. Jour. Physiol. 72: 583–594.
(24) MELVIN, ROY.
1931. A QUANTITATIVE STUDY OF COPPER IN INSECTS. Ann. Ent. Soc. Amer. 24: 485–488.
(25) MENDEL, BRUNO, and GOLDSCHEIDER, INGEBORG.
1925. EINE KOLORIMETRISCHE MIKROMETHODE ZUR QUANTITATIVEN BESTIMMUNG DER MILCHSÄURE IM BLUT. Biochem. Ztschr. 164: [163]–174, illus.
(26) MUTTKOWSKI, RICHARD A.
1921. STUDIES ON THE RESPIRATION OF INSECTS. I. THE GASES AND RESPIRATORY PROTEINS OF INSECT BLOOD. Ann. Ent. Soc. Amer. 14: 150–156.
(27) PATTERSON, JOCELYN.
1928. DETERMINATION OF CHLORIDES IN BODY FLUIDS. Biochem. Jour. 22: [758]–761.
(28) PETERS, JOHN P., and VAN SLYKE, DONALD D.
1932. QUANTITATIVE CLINICAL CHEMISTRY. v. 2. Baltimore.
(29) SHOHL, ALFRED T., and BENNETT, HELEN B.
1928. A MICRO METHOD FOR THE DETERMINATION OF POTASSIUM AS IODOPLATINATE. Jour. Biol. Chem. 78: 643–651.
(30) SOMOGYI, MICHAEL.
1926. NOTES ON SUGAR DETERMINATION. Jour. Biol. Chem. 70: 599–612, illus.

(31) STEDMAN, ELLEN, and STEDMAN, EDGAR.
1925. HAEMOCYANIN. PART I. THE DISSOCIATION CURVES OF THE OXYHAEMOCYANIN IN THE BLOOD OF SOME DECAPOD CRUSTACEA. Biochem. Jour. 19: [544]–551, illus.

(32) TISDALL, FREDERICK F.
1922. A RAPID COLORIMETRIC METHOD FOR THE QUANTITATIVE DETERMINATION OF THE INORGANIC PHOSPHORUS IN SMALL AMOUNTS OF SERUM. Jour. Biol. Chem. 50: 329–337.

(33) VOIT, CARL.
1891. UEBER DIE GLYKOGENBILDUNG NACH AUFNAHME VERSCHIEDENER ZUCKERARTEN. Ztschr. Biol. 28: [245]–292.

(34) WARBURG, OTTO.
1927. METHODE ZUR BESTIMMUNG VON KÜPFER UND EISEN UND ÜBER DEN KÜPFERHALT DES BLUTSERUMS. Biochem. Ztschr. 187: [255]–271 illus.

(35) WIGGLESWORTH, V. B.
1931. THE PHYSIOLOGY OF EXCRETION IN A BLOOD-SUCKING INSECT, RHODNIUS PROLIXUS (HEMIPTERA, REDUVIIDAE). Jour. Expt. Biol. 8: 411–451, illus.

(36) WINTERSTEIN, HANS.
1909. ZUR KENNTNIS DER BLUTGASE WIRBELLOSER SEETIERE. Biochem. Ztschr. 19: [384]–424.

(37) YEAGER, J. FRANKLIN, and KNIGHT, HARRY H.
1933. MICROSCOPIC OBSERVATIONS ON BLOOD COAGULATION IN SEVERAL DIFFERENT SPECIES OF INSECTS. Ann. Ent. Soc. Amer. 26: 591–602.

THE BIOLOGICAL DISPOSITION OF ROTENONE AFTER INGESTION BY THE SOUTHERN ARMYWORM [1]

By P. A. Woke

Assistant entomologist, Division of Control Investigations, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture

INTRODUCTION

The toxic action of ingested rotenone has been observed to vary markedly with different species of insects. Campbell [2] in 1929 (*8*) [3], using the sandwich method described by Campbell and Filmer (*2*), determined the median lethal dose for fourth-instar silkworms (*Bombyx mori L.*) to be near 0.003 mg per gram of body weight. Hansberry and Richardson (*4*) state that this value was twice duplicated in their laboratory, and that the same value had been obtained by Kagy. They also report a median lethal dose for *Vanessa cardui* (L.) of 0.03 mg per gram. Swingle (*9*) reports that cabbage worms died within 24 hours after eating very small quantities of a gelatin film containing derris. There are indications [2] that rotenone may be toxic in similar small dosages to several other species of insects. There are also indications [2] that ingested rotenone exerts little or no toxic action on certain other species. Hansberry and Richardson (*4*) found that a dose of 0.49 mg per gram was apparently nontoxic to *Heliothis obsoleta* (F.). This dose, they point out, is 16 times the median lethal dose for *Vanessa cardui* and 160 times that for *Bombyx mori*. The high specificity of this toxicant suggests a relationship to differences in the physiological characteristics of the different species of insects and to their food (*8*).

According to Lightbody and Mathews (*7*), the toxicity of rotenone to white rats is increased by feeding it dissolved in oil, or by feeding oil following rotenone.

Ambrose and Haag (*1*) report that their studies on the toxicity of derris to mammals indicate that part or all of the rotenone ingested may be absorbed and slowly excreted into the intestinal tract, and that it is eliminated in the feces either free or in some form closely resembling rotenone. Species difference in toxic action was observed.

The present investigation was undertaken to determine the biological disposition of rotenone after ingestion by the southern armyworm (*Prodenia eridania* (Cram.)).

Preliminary experiments had shown that sixth-instar larvae of the southern armyworm ingest turnip-leaf sandwiches containing 5mg of rotenone as readily as they do similar sandwiches without rotenone, and that healthy larvae apparently suffer no ill effects from the ingestion of this quantity of rotenone. There was some evidence, however, that toxic action may be produced under certain, perhaps abnormal, conditions.

[1] Received for publication May 19, 1938; issued November 1938.

[2] CAMPBELL, F. L. REVIEW OF INFORMATION ON THE INSECTICIDAL VALUE OF ROTENONE. U. S. Bur. Ent. Mimeo. Cir. E-298, 28 pp. 1932.

[3] Italic numbers in parentheses refer to Literature Cited, p. 712.

MATERIALS AND METHODS

The larvae used in these experiments were sixth instars of the southern armyworm reared in a greenhouse insectary on turnip plants and cut lettuce. The rotenone used was as nearly pure as could be determined by chemical examination.[4]

Toxicity was determined by the use of mosquito larvae (*Culex fatigans* Wied.) as biological indicators according to the method reported by Campbell and associates (*3*, *5*). This method has been used successfully for the determination of comparative toxicity of a number of insecticidal substances, including rotenone (*5*), to which mosquito larvae are rather sensitive.

The rotenone was fed to the armyworm larvae in sandwiches, each of which was prepared as follows: Disks 22 mm in diameter were cut from turnip leaves. One disk was lightly coated on the smooth side with starch paste, and 5 mg of rotenone was distributed evenly over the surface. A second disk, also lightly coated with starch paste on the smooth side, was caused to adhere to the first disk with the rotenone between.

The larvae were removed from food several hours before being offered the sandwiches. Each larva was placed in a 150-ml beaker with one sandwich and kept for 18 hours overnight. In the morning all the larvae that had ingested entire sandwiches were selected and were conserved on fresh turnip leaves 24 hours longer. It was assumed that this period of time would be ample for the complete evacuation from the alimentary canal of all the rotenone or rotenone derivatives that might be eliminated.

The presence of unaltered rotenone in the tissues, in the contents of the gut, or in the feces of the larvae would be detectable by testing acetone extracts of these materials against mosquito larvae used as biological indicators. Accordingly the materials were prepared for this purpose. All the feces egested by all the armyworm larvae during the 42-hour period were collected and combined. The armyworm larvae were dissected, and the contents of the guts of all the larvae were removed and combined. The tissues of all the larvae were also combined. Each of the three materials was ground with sand, desiccated at 50° C. in a partial vacuum, reground, and extracted four times at room temperature with 50 ml of acetone. Satisfactory extraction was obtained by this method, as indicated by the application to the marc of the color test for rotenone (*6*).

By experiment it was determined that 0.2 ml of acetone per milligram of rotenone is well above the minimum quantity required to hold the rotenone in solution, and that this quantity of acetone, without rotenone, in 0.1 percent gelatin solution is not toxic to mosquito larvae in the lowest dilution employed, as described below. It was also determined that, if impurities were present in the acetone, they were not toxic to mosquito larvae even when concentrated by the evaporation of a large quantity of acetone. On the basis of these facts, the excess acetone was eliminated by evaporation at 25°–30° C. in a partial vacuum, leaving the final volume of acetone as 0.2 ml for each milligram of rotenone originally ingested by the larvae.

[4] A sample of the rotenone was analyzed for purity by C. C. Cassil, of the Division of Insecticide Investigations of this Bureau. The Gross-Smith color test showed 98 percent purity, and the optical rotatory power, $[\alpha]_D^{20°}$, was found to be −222.5°, as compared with −220.8° given by pure rotenone.

Each of the three extracts that were prepared as outlined above was tested against mosquito larvae to determine the presence of a substance toxic to these insects. The absence of such toxic substance would indicate the absence of rotenone, while the presence of such would require its identification as rotenone or some other toxic substance, probably a rotenone derivative. Each of the three extracts was placed in distilled water containing 0.1 percent of gelatin. On the assumption, which proved correct, that all the rotenone ingested by the larvae might be in one extract, dilutions of the extracts at the rate of 1 part of rotenone to 100,000, 500,000, 750,000, and 1,000,000 parts of gelatin solution were prepared, One hundred milliliters of each of the four dilutions of each of the three extracts (12 samples) was placed in each of 12 Erlenmeyer flasks. Twelve lots of 100 mosquito larvae each were prepared, and one lot was placed in each flask. The mortality was determined after exposure for 20 hours at 29° C. in the dark.

The tests, which were prepared as outlined above, were accompanied by three checks. The term "test" as here used refers to the diluted extracts of material prepared from southern armyworm larvae that had ingested rotenone. One check consisted of diluted extracts of materials prepared from armyworm larvae that had not ingested rotenone and to which rotenone had not been added. The purpose of this check was to determine the possible toxic influence of the extracts of the larval materials alone on the mosquito larvae. The second check was like the first except that a quantity of rotenone equal to that ingested by the larvae from which the tests were prepared was mixed with each of the three materials—feces, contents of the gut, and tissues of the larvae—at the time the materials were ground before extraction. The purpose of this check was to determine the toxic influence of a known quantity of rotenone added to the larval materials. A third check consisted of dilutions of a gelatin solution of a quantity of rotenone equal to that ingested by the armyworm larvae from which the tests were prepared. Its purpose was to determine the toxic action of a known quantity of rotenone without the larval materials. In all respects except as indicated the checks were prepared in precisely the same manner as were the tests.

The arrangement as outlined above constituted one trial, and was carried out three times to increase the reliability of the results. Essentially the same results were obtained in all three trials.

From 7 to 10 armyworm larvae were used for the preparation of the test materials in each trial, or a minimum of 24 larvae for the three trials. In each trial the same numbers of larvae were used in the preparation of each check that were prepared from armyworm materials. A minimum of 72 larvae were therefore used altogether in the preparation of the tests and two checks.

RESULTS

The results are summarized in table 1. Each figure is the average mortality of the 1,200 mosquito larvae in all four dilutions and in all three trials.

From an examination of these figures it is apparent that the extracts of the tissues and of the gut contents of rotenone-fed larvae possessed practically no toxicity, while the extract of the feces of the same

larvae were highly toxic. The possible influence of the extracts of the larval materials alone on the toxicity may be disregarded, since the negligible mortality in the no-rotenone check indicates that they possess little or no toxicity. The mortality in the second check is high and indicates approximately the mortality that might be expected should all the rotenone ingested by the armyworm larvae appear in any one of the three test materials. Rotenone in gelatin solution without larval extracts gave similar high mortality.

TABLE 1.—*Toxicity to mosquito larvae of acetone extracts of tissues, gut contents, and feces of southern armyworm larvae that had ingested rotenone, and of similar extracts of larvae that had not ingested rotenone when rotenone was and was not added before extraction*

Acetone extracts of materials from armyworm larvae	Average mortality of mosquito larvae exposed to—		
	Extracts of armyworm larvae that had ingested rotenone (tests)	Extracts of armyworm larvae that had not ingested rotenone (checks)	
		No rotenone added before extraction	Rotenone added before extraction
	Percent	*Percent*	*Percent*
Larval tissues (gut contents removed)	0.75	0.33	86.00
Gut contents	.91	.17	85.25
Feces	82.50	1.17	85.41
Gelatin solution without larval extract (check)			89.00

The practical absence of mortality of mosquito larvae exposed to extracts from tissues and gut contents of larvae that had ingested rotenone indicates that rotenone is not present in the tissues of larvae or in the contents of the digestive tract 24 to 42 hours after ingestion of the substance, at least in quantities sufficient to be detected by the method used.

The toxicity of the fecal extracts of rotenone-fed larvae was similar to the toxicity of the fecal extract of the rotenone-added check and also of the rotenone-only check, and indicates the presence of rotenone or of some other toxic agent.

Microscopic examination of the feces of larvae that had ingested rotenone revealed the presence of quantities of apparently unaltered particles, assumed to be those of rotenone by their similarity to the same material before ingestion and by their absence from feces of larvae that had not ingested rotenone. The occurrence of toxicity in feces of larvae that have ingested rotenone equivalent to that in feces of normal larvae to which a quantity of rotenone equal to that ingested by the first group of larvae has been added probably indicates, if no chemical change has occurred, that rotenone ingested by these larvae is wholly or largely eliminated in the feces.

Since the known derivatives of rotenone that are at all likely to be producible in the gut of the insect are nontoxic or of decidedly lower toxicity than rotenone, and since the color test for rotenone (*6*) was observed, it is probable that chemical change has not occurred and that the observed toxicity is due to the presence of rotenone.

Preliminary experiments in which the desiccation of the larval

material, the extractions, and the evaporation of the acetone were performed with heat at atmospheric pressure gave similar results. The reduced-pressure method finally used, however, prevents the destruction of the rotenone or reduction of its toxicity due to heat or to prolonged boiling in solution.

SUPPLEMENTARY EXPERIMENTS

DISPOSITION OF INGESTED ROTENONE AS DETERMINED BY CHEMICAL METHODS

To substantiate the biological evidence with chemical evidence, feces from 11 larvae that had ingested 5 mg of rotenone each were tested [5] for the possible identification of rotenone and determination of the quantity that passed through the insect unchanged. By the Gross-Smith colorimetric method of estimating rotenone, 45 mg of rotenone was found in the entire feces and 2 mg was found in the acetone washings of the beakers in which the feces had been collected. This is a total of 47 mg out of the 55 mg that had been fed to the 11 larvae. That the material in the feces was really rotenone was determined by the fact that it gave the Durham blue color test, and more definitely by the fact that crystals obtained from the extract had the common habit of rotenone crystals and showed certain optical properties, that is, parallel extinction and alpha and gamma indices of refraction, the same as pure rotenone.

EFFECTS OF ISOLATED LARVAL TISSUES ON THE TOXICITY OF ROTENONE

Another experiment was conducted to determine whether the toxicity of rotenone would be affected by incubation with any of the separate isolated tissues or the gut contents of the southern armyworm. The blood, skin, tissues of the digestive tract and the malpighian tubules, muscular tissues, fat body, contents of the foregut and the hindgut, and contents of the midgut were isolated from 10 healthy larvae and corresponding kinds of materials combined. Five milligrams of rotenone was mixed with each kind of material, which was then ground with sand in a mortar. The thoroughly mixed and ground materials were incubated in the dark for 18 hours at 29° C., desiccated, extracted with acetone, and tested against mosquito larvae for the presence of toxic principles as previously described. Corresponding materials from 10 other healthy armyworm larvae were treated in the same manner, except that rotenone was not mixed with them, to serve as checks for determination of the possible toxic effects of the larval material alone on the mosquito larvae. An acetone solution of rotenone served to demonstrate the toxic effects of rotenone alone.

The mosquito larvae appeared to be unaffected by the extracts of the larval tissue and the contents of the digestive tract that had been incubated without rotenone, as indicated by the low mortality, which averaged only 0.83 percent. The mortality of the mosquito larvae in the tests in which rotenone was incubated with the various tissues and with the contents of the digestive tract averaged 98 percent, corresponding closely to that of the rotenone-only check, which averaged 99.3 percent. The results of this experiment indicate that little

[5] These analyses were made by C. C. Cassil and E. L. Gooden, of the Division of Insecticide Investigations.

or no reduction in toxicity of rotenone occurs during incubation for 18 hours in the dark at 29° C. with any of the tissues or with the gut contents.

SUMMARY AND CONCLUSIONS

Experiments were made to determine the biological disposition of rotenone after ingestion by the southern armyworm (*Prodenia eridania* (Cram.)). Sixth-instar larvae that had been reared on turnip plants and cut lettuce were used. Rotenone was fed to the larvae in sandwiches, and after intervals of time acetone extracts of the tissues, gut contents, and feces were prepared and tested against mosquito larvae for the determination of toxicity. Suitable checks were employed.

The results show that the southern armyworm larva, after ingesting 5 mg of rotenone, eliminates all or most of the substance with its feces. This result was substantiated by chemical tests.

It was also shown that the toxicity of finely powdered rotenone to mosquito larvae is not altered to a demonstrable extent after 18 hours' incubation in the dark at 29° C. with the various tissues or with the contents of the digestive tract.

LITERATURE CITED

(1) Ambrose, Anthony M., and Haag, Harvey B.
1937. Toxicological studies of derris. Comparative toxicity and elimination of some constituents of derris. Indus. and Engin. Chem. 29: 429–431.

(2) Campbell, F. L., and Filmer, R. S.
1929. A quantitative method of estimating the relative toxicity of stomach-poison insecticides. 4th Internatl. Cong. Ent. Ithaca, August 1928, Trans. v. 2, pp. 523–533, illus.

(3) Campbell, F. L., Sullivan, W. N., and Smith, C. R.
1933. The relative toxicity of nicotine, anabasine, methyl anabasine, and lupinine for culicine mosquito larvae. Jour. Econ. Ent. 26: 500–509, illus.

(4) Hansberry, T. Roy, and Richardson, Charles H.
1936. Toxicity of certain stomach poisons to several common lepidopterous larvae. Jour. Econ. Ent. 29: 1160–1166.

(5) Jones, Howard A., Gersdorff, W. A., Gooden, E. L., Campbell, F. L., and Sullivan, W. N.
1933. Loss in toxicity of deposits of rotenone and related material exposed to light. Jour. Econ. Ent. 26: 451–470, illus.

(6) Jones, Howard A., and Smith, Charles M.
1933. A color test for rotenone. Indus. and Engin. Chem., Analyt. Ed. 5: 75–76.

(7) Lightbody, Howard D., and Mathews, Joseph A.
1936. Toxicology of rotenone. Indus. and Engin. Chem. 28: 809–811.

(8) Shepard, H. H., and Campbell, F. L.
1932. The relative toxicity of rotenone and some related compounds as stomach insecticides. (Sci. Note) Jour. Econ. Ent. 25: 142–144.

(9) Swingle, M. C.
1934. Difference in action of derris and pyrethrum against the imported cabbage worm shown by experiments with gelatin films containing these plant materials. (Sci. Note) Jour. Econ. Ent. 27: 1101–1102.

U. S. GOVERNMENT PRINTING OFFICE: 1938

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 57 WASHINGTON, D. C., NOVEMBER 15, 1938 No. 10

SAMPLING ERROR IN TIMBER SURVEYS[1,2]

By A. A. HASEL,

Associate silviculturist, California Forest and Range Experiment Station,[3] Forest Service, United States Department of Agriculture

INTRODUCTION

In cruising timber, the mean volume of any given area is usually estimated from the mean volume per unit area of a small percentage of the timberland taken as a sample. This estimated mean volume will inevitably differ to some extent from the true mean volume. With the elimination of such factors as bias in measurement of trees and of areas or use of inappropriate volume tables, the probable range of difference between true mean volume and estimated mean volume is dependent upon sampling error alone. If the plots or sampling units making up the sample meet the conditions of independent and random selection, the sample itself contains the information needed for estimating sampling error.

While it is of interest to know with what precision the estimated mean has been found, it is vitally more important from an economic standpoint to be able to predetermine what shall be a sufficient sample for an assumed allowable range of error. If the sampling error is not already known, it is necessary to take a preliminary sample of the area to gain this information. From this, it is possible to establish the intensity of the cruise that will produce results within the assumed allowable range of error of the mean. It is then necessary only to provide for occasional supplementary checks, as the cruise progresses, for adequacy of the work done.

In regular cruising practice a systematic arrangement of plots is used in which the sample is made up of contiguous plots forming equidistant strips, or of plots taken at regular intervals along equidistant lines. In most timber surveys, cover type and topographic maps are made in conjunction with the cruise, and for this purpose it is desirable that cruise lines be spaced equidistant in order that all parts of the area may be mapped to a satisfactory standard. As stand and type variation are generally greatest at right angles to contour lines, the cruise lines are usually run in that direction, and plots are spaced closer along lines than between lines. By this procedure type and contour lines are more readily located and mapped, and the more intensive sampling in the direction of greatest variation improves the accuracy of volume estimates.

This arbitrary spacing of plots presents difficulties, should a test of adequacy of sampling be attempted by treatment as a random sample in statistical analysis, since it violates the basic requirement that each possible plot in an area have an equal and independent

[1] Received for publication April 26, 1938; issued December 1938.
[2] Appreciation is extended to the Works Progress Administration for assistance rendered by statistical workers employed on Project No. 365-03-3-35.
[3] Maintained at Berkeley, Calif., in cooperation with the University of California.

chance of selection in sampling. Experiments in field crops have shown time and again that there is systematic variation in yield from one part of an area to another. Adjacent or neighboring plots tend on the average to be more alike than plots farther apart. Without previous knowledge of an area, it should be assumed that a population of plots is more or less heterogeneous, regardless of appearance of uniformity. The only method of assuring that the elements contributing to heterogeneity are represented in the sample in about their true proportion is to select at random the plots or parts of a sample contributing the estimate of sampling error. The plots indicated for cruising may, for example, be selected by drawing from thoroughly mixed numbers designating each possible plot location. After each draw, the numbered slip is returned and the numbers mixed before another draw. Repeats of plot numbers are rejected, as the objective is a fixed percentage of total area in the sample with each plot selected contributing the same amount of information per unit of area. An improvement over this method, assuring the same freedom of selection, is the use of Tippett's random sampling numbers (*11*).[4] The locating of such plots on the ground may be less convenient than with systematically spaced plots and may also make necessary the running of additional line for mapping. This is the sacrifice necessary for assurance of a valid estimate of error.

The fact that the sampling of timber stands, except for technique in collection of data, is essentially the same as any other problem in sampling in which soil heterogeneity is likely to be present, has not been fully recognized. Apparently advantage has not been taken of methods of testing for heterogeneity and, if indicated, of eliminating its effect on estimates of sampling error—methods first proposed and described by Fisher and Mackenzie (*3*) in 1923. Areas considered as of the same timber type, condition class, age class, and site quality have been treated as homogeneous populations. Various arbitrary plot spacings have been used. Mudgett and Gevorkiantz (*6*) in estimating the reliability of forest surveys in Wisconsin used plots at one-eighth mile intervals along parallel lines 1, 2, or 3 miles apart. Within the break-downs made in a type the errors of random sampling were based on total variation between systematically arranged plots. In the bottom-land hardwood forest survey, Schumacher and Bull (*9*) based their estimate of error on plots at one-eighth mile intervals along lines 3 miles apart. The plots were grouped according to forest condition, i. e., virgin, cut-over, second growth, etc., and the weighted mean of the standard deviations so obtained was used in estimating sampling error. In New England, Goodspeed (*4*) compared line-plot survey, using plots at 165-foot intervals along lines 330 feet apart, with continuous-strip survey along the same lines, taking each 165-foot length of strip as an independent observation. Since the strips and plots overlapped 44.5 percent, variation in sampling was actually confined to 55.5 percent of each sample. From statistical analysis of the data he concluded that the two methods, when applied with equal precision, gave essentially similar and equally reliable results. Preston (*7*) recommended statistical analysis of systematic line-plot cruises and suggested that timber types be mapped separately and prior to sampling. He pointed out the futility of adhering to a 5- or 10-percent, or any other fixed, preconceived intensity of cruise.

[4] Italic numbers in parentheses refer to Literature Cited, p. 735.

In Canada, Wright (*12*) used plots at 10-chain intervals along lines 10 chains apart. In strip survey his strips were run at half-mile intervals, and the tally kept separately by 2-chain segments. Seven estimates were made by taking the first 6 chains in each half mile of strip as a plot, the next 6 chains for a plot in the second sample, and so on to the seventh sample which was made up of plots 4 chains in length. The average of these seven sample estimates of variation was applied to the mean of all plots in calculating sampling error. Wright stated that the main requirement in the use of statistical methods in examining the accuracy of an estimate was to have a reliable figure for standard deviation. Robertson (*8*) favored small plots evenly distributed over the area as against a few hand-picked large plots, with analysis of data from time to time during the survey to insure the taking of sufficient plots to give an accuracy within the required limits for any given factor. The line-plot arrangement he found to be more accurate than the strip. Since in strip survey the plots are contiguous in one direction, he suggested that they represented conditions in that direction beyond their due proportion. Candy (*1*) stated that any method of survey for which it is possible to calculate the accuracy of the estimate obtained, is very much superior to methods in which the accuracy of estimate is doubtful and not at all calculable. He used both systematic strip and line plot, and concluded that only with line plots could adequacy of sampling be determined.

In the present study the true mean volume and the true variation between plots are known for an area of 5,760 acres. The distribution of plot volumes is known to approach that of the normal frequency distribution. Thus all of the essential statistical information regarding the population is at hand. The expected range of sample means from the true mean is calculated, taking into account the effect of such factors as size, shape, arrangement of plots, and intensity of sampling. From samples taken according to the specifications set up, the observed range of means from the true is compared with the expected range. Checks of theoretical with actual efficiency of sampling methods are readily made, since the timbered area studied is part of the Blacks Mountain experimental forest, in which the total timbered area of 9,078 acres has been completely inventoried and plot locations, cover types, and topography mapped. The usual assumption that systematically spaced plots do fulfill the requirements of independent and random selection is tested, both for strip and line-plot arrangements. Efficiency of different methods of cruising is determined on the basis of relative intensity of sampling required for a given accuracy of estimate. For cruises giving valid estimates of sampling error, the precision of sample estimates of population variance is given.

MATERIAL AND METHODS

The 5,760 acres of virgin timber on which the analyses are based is located in northeastern California, within the Lassen National Forest. The timber type is classed as pure pine, with more than 90 percent of the volume in ponderosa pine (*Pinus ponderosa* Dougl.) and Jeffrey pine (*P. jeffreyi* Oreg. Com.). The other timber species are white fir (*Abies concolor* Lindley and Gordon) and incense cedar (*Libocedrus decurrens* Torr.). The stand is all-aged, with young and overmature age classes well represented, but is understocked owing to light representation in the intervening age classes. With the exception of

small areas, the stand over the entire area appears uniform to the eye and not stratified according to any of the criteria used in mapping such stands. In other words, it appears to be a fairly homogeneous population.

The nine sections shown in figure 1 were selected for study because they were full sections and, with the exception of small areas, completely timbered. Board-foot volumes of trees 12 inches and more in diameter were totaled for the 2,304 individual 2.5-acre plots contained in the nine sections. These plots, rectangular in shape (2.5 by 10 chains), are designated as basic plots. In addition, because their

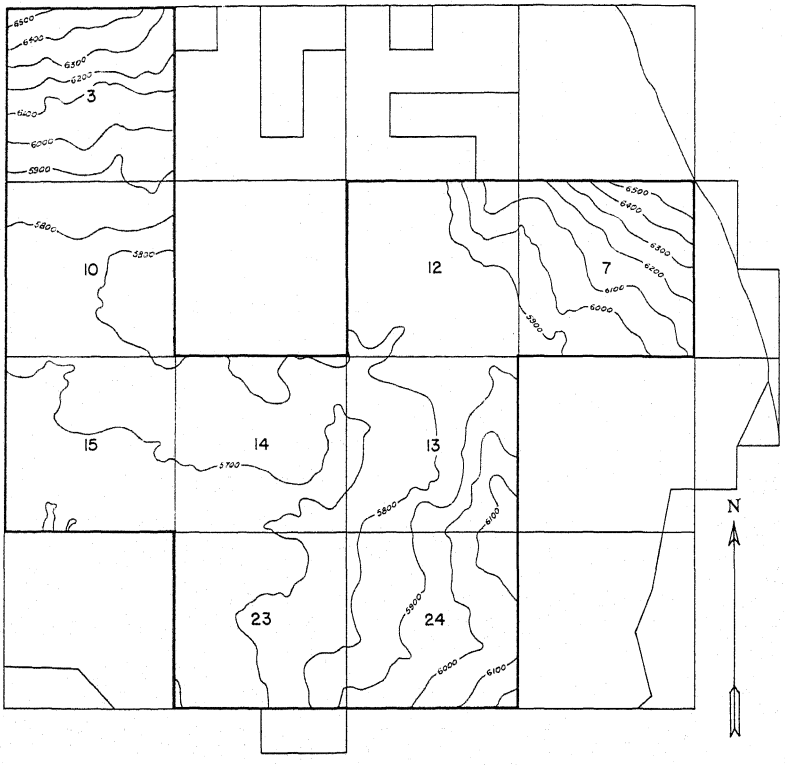


FIGURE 1.—Contour lines and section numbers of area selected for analysis from the Blacks Mountain Experimental Forest.

locations are mapped, adjacent plots may be combined into totals of 1,152 5-acre plots (5 by 10 chains), 576 10-acre plots (10 by 10 chains), or other shapes and sizes in multiples of the basic plot size and dimensions.

Some irregularity in size and shape of basic plots along section lines was unavoidable owing to variation of sections from exact mile squares. For these plots the volumes were proportionately reduced or increased to the 2.5-acre basis. The adjustments needed amounted to a negligible percent of the total. All basic plots were taken with the long axis in an east-west direction.

Subdivisions larger than plots are termed blocks, and are usually taken as regular Land Office subdivisions such as forties, quarter

sections, half sections, or sections. Figure 2 shows the size and shape of plots and blocks mainly used. When a plot making up part of a sample is selected independently and at random it constitutes a random sampling unit. When plots are spaced at regular intervals along lines, and the lines selected independently and at random, the line-plot combination is the sampling unit. Likewise, where plots are contiguous end to end forming strips and the strips are selected independently and at random, the strip constitutes the sampling unit. The resulting arrangements may be termed random line plot and random strip, respectively. When the sampling units are taken at

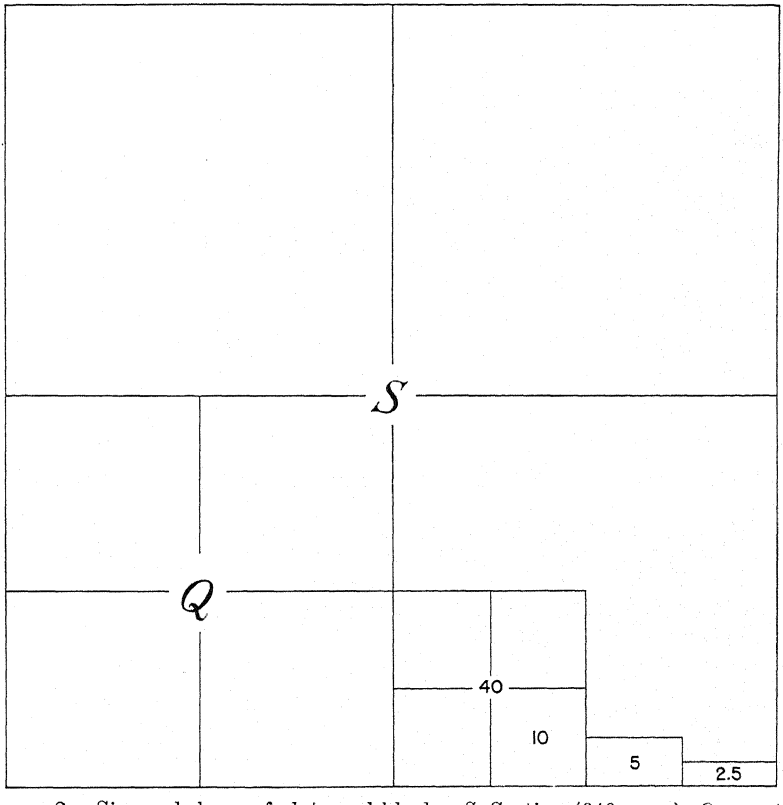


FIGURE 2.—Size and shape of plots and blocks: *S*, Section (640 acres); *Q*, quarter section (160 acres); smaller units designated by area in acres.

random from all possible in the population, the sampling is referred to simply as random, as contrasted to selection of equal numbers of units from each block, which is termed "*random within blocks.*" Systematic strip and line-plot arrangements are representative samples in that each block is sampled to the same intensity, but are not random.

The total variation between random sampling units is apportioned into parts contributed by various known sources and the error variance segregated by the analysis of variance method of Fisher. Descriptions of this method and others used in the study are given by Fisher (*2*, *3*) and Snedecor (*10*), and an instructive application of the method by Ivanov (*5*).

RESULTS

SIZE AND SHAPE OF PLOT

The essential information concerning stand variability is given in table 1. The mean square ratios all exceed F at 0.01 as given by Snedecor (*10*), and therefore volume is correlated with place and the population is heterogeneous. In general, variation between plot

TABLE 1.—*Analysis of variance*

Source of variation	Sum of squares	Degrees of freedom	Mean square	F [1]	F at 0.01 [2]
Sections from general mean	109,693.3526	8	13,711.6691	6.39	3.26
Quarter sections within sections	57,937.4032	27	2,145.8297	3.48	1.98
Forties within quarter sections	66,569.5344	108	616.3846	1.65	1.11
Tens within forties	161,109.7722	432	372.9393	1.76	1.08
Fives within tens	121,940.8201	576	211.7028	1.83	1.04
Basic plots within fives	133,337.7395	1,152	115.7446		
Total, basic plots from general mean	650,588.6220	2,303	[3] 282.4961		

[1] $F=\frac{\text{larger mean square}}{\text{smaller mean square}}$.

[2] As given by Snedecor (see text). Values equal to or exceeding these indicate that differences are highly significant statistically.

[3] $\sqrt{282.4961}=16.81$=standard deviation of a basic plot from the population mean of 40.83 thousand feet board measure.

volumes tends to increase as the distance between plots is increased. For this reason, size and shape of plot will be an important factor in efficiency of sampling. Two plots taken side by side will in general include less of the stand variation within their combined area and more will remain between such pairs than will be the case with pairs of separated plots. Thus variation between 5-acre plots will be greater than variation between 5-acre sampling units each made up of two 2.5-acre plots spaced apart along a line. Likewise, owing to correlation between adjacent plots, variation between 5-acre plots, expressed in terms of single 2.5-acre plots taken as parts of 5's, will be greater than variation between 2.5-acre plots as random sampling units. Less stand variation per unit of area is included within 5-acre plots as sampling units than within 2.5-acre sampling units.

The mean square between 2.5-acre plots in table 2 is the total mean square from table 1. It is the error variance of randomly selected 2.5-acre plots. The corresponding value for 5-acre plots is obtained from table 1 by pooling sums of squares and degrees of freedom from 5's within 10's up to and including sections from general mean. The quotient of these two summations—449.3926—is the mean square of randomly selected 5-acre plots, expressed in terms of single 2.5-acre plots. This is considerably larger than 282.4961, and the effect on sampling efficiency is evident from the following formula for variance of a mean:

$$\sigma_M^2=\frac{\sigma^2}{n} \qquad (1)$$

in which

σ_M^2=the variance of a sample mean, or the standard error squared
σ^2=the variance, or mean square, of the basic plots
n=the number of basic plots.

For a given standard error, n will need to be 449.3926/282.4961=1.59 times as great with 5-acre plots as with 2.5-acre plots. In random sampling from an unlimited population, 1.59 times as much land is needed in sample area with 5-acre plots as with 2.5-acre plots to assure the same precision.

Stated in another way, 282.4961/449.3926=62.86 percent, the efficiency of 5-acre plots as compared to 2.5-acre. To equal in precision the estimate of volume based on sampling with 5-acre plots, an area only 62.86 percent as large would be needed with the smaller plots. The advantage of 2.5-acre plots over 10-acre plots is still greater.

When plots and sampling units larger than the basic plots are used, the variance is then weighted according to the number of basic plots in the larger plot. Likewise n is in terms of basic plots. Obviously, the resulting standard error obtained is not affected. To keep mean squares directly comparable regardless of size, shape, or arrangement of plots used, they are expressed in terms of single basic plots throughout this article.

It is evident from table 2 that, among the 10-acre plots, the long, narrow shape is the most efficient. This is due partly to the presence of small nontimbered areas of such shape that several 10 by 10 and 5 by 20 plots contained little or no volume and consequently increased the average variation. The effect of placing of plots with respect

TABLE 2.—*Relative efficiency of plots of varying size and shape in the use of land*

Plot size and shape	Mean square	Efficiency	Relative size of sample for a given precision of estimate
		Percent	
2.5-acre: 2.5 by 10 chains [1]	282.4961	100.00	1.00
5-acre: 5 by 10 chains	449.3926	62.86	1.59
10-acre:			
2.5 by 40 chains	[2] 566.0602	49.90	2.00
5 by 20 chains	[2] 711.8291	39.68	2.52
10 by 10 chains	687.4958	41.09	2.43

[1] 1 chain=66 feet.
[2] Obtained by separate calculation of population variance. All other mean squares obtained from table 1 directly by pooling degrees of freedom and sums of squares.

to direction of greatest stand variation is illustrated in figure 1. Variation in timber stands is usually greatest at right angles to the contour lines. In collecting the present data, the longer plot axis always extended in an east-west direction. Thus, within sections having marked changes in elevation, sections 24 and 13 were cruised with plot length in the direction of greatest variation, section 7 at a 45-degree angle to greatest variation, and section 3 parallel to greatest variation. In this respect it would seem that sampling by sections about balances. Had length always been taken at right angles to the contours, the advantages of long, narrow plots would be greater, and possibly the 5 by 20 plots would appear as superior to the 10 by 10.

It should be emphasized that the results in table 2 are based on random sampling from a theoretically infinite parent population. In sampling methods discussed later, where corrections are made to the mean of the limited population and variation between blocks

eliminated, these relative efficiencies will not be exactly true, although quite close.

RANDOM PLOT AND RANDOM-WITHIN-BLOCK CRUISES

As previously stated, in sampling a heterogeneous population it is necessary to select the sampling units independently and at random to insure that the heterogeneous elements are represented in about their true proportion. Using 2.5-acre plots as sampling units, random sample estimates of variance will tend to approach 282.4961, the true variance. As the number of sample estimates is increased, their average will approach more closely to the true, and likewise, estimates from larger samples will be grouped closely about the true variance and closely in accord with the normal law of frequency of error. A valid sample estimate of variance may be substituted in formula (1), in which n is known and the standard error calculated. This tells us the range from the sample mean within which the chances are 2 in 3 that the true or population mean lies. By doubling the standard error obtained we have the range for which the chances are 19 in 20.

With intensive sampling, formula (1) gives appreciable overestimates of sampling error because it estimates the range from the theoretical infinite parent-population mean while here we are interested in range from a parent population limited to 2,304 2.5-acre plots. By substituting 2,304 as n in the formula we can get the estimated range of the population mean from the limited population mean. This is the irrevelant portion of total variance of sample means and is represented by $\frac{\sigma^2}{N}$ in the following formula:

$$\sigma_M{}^2=\frac{\sigma^2}{n}-\frac{\sigma^2}{N} \qquad (2)$$

in which

N=the number of 2.5-acre plots in the limited population (a constant=2,304).

In practice a single estimate of variance together with the sample mean are the two statistics supplied. With the present data the true variance and mean are known for the limited population, and by varying n in formula (2) the expected grouping of sample means about the true mean can be calculated for different intensities of sampling. Since σ^2 changes with varying size and shape of plot, the effect of these factors is also reflected in the expected grouping of sample means. The appropriate mean squares for substitution as σ^2 in formula (2) are given in table 3, the values for which are derived from table 1. The expected standard errors for random samples of varying intensity, made up of plots of varying size, are given in table 4. In all, the expected standard errors based on true variance are shown for seven such cruises by the random arrangement of plots. For each cruise, four independent samples were drawn to compare expected values with observed values. The 2,304 plots were numbered consecutively and the required number of plots for each cruise selected by use of Tippett's (*11*) random sampling numbers. The sample

estimates of standard error and deviations from the true mean are shown in table 4 along with the corresponding true standard error.

TABLE 3.—*Variance of plots from the general mean and from block means*

Plot size and source of variation	Sum of squares	Degrees of freedom	Mean square [1]	Variance of plot means [2]	Standard deviation
2.5-acre:					
From general mean	650,588.6220	2,303	282.4961	282.4961	16.81
Within sections	540,895.2694	2,295	235.6842	235.6842	15.35
Within quarter sections	482,957.8662	2,268	212.9444	212.9444	14.59
Within forties	416,388.3318	2,160	192.7724	192.7724	13.88
5-acre:					
From general mean	517,250.8825	1,151	449.3926	224.6963	14.99
Within sections	407,557.5299	1,143	356.5683	178.2842	13.35
Within quarter sections	349,020.1267	1,116	313.2797	156.6398	12.52
10-acre:					
From general mean	395,310.0624	575	687.4958	171.8739	13.11
Within sections	285,616.7098	567	503.7332	125.9333	11.22
Within quarter sections	227,679.3066	540	421.6283	105.4071	10.27

[1] Variance of a single basic (2.5 acre) plot.
[2] Variance of the mean of basic plots making up larger plots.

TABLE 4.—*Expected and observed deviations of sample means from the true mean volume* [1]

Plot and sample size	Row No.	Random sampling from total population			Random sampling within blocks [2]		
		Standard error based on population variance	Standard error based on sample estimate of population variance	Observed deviation of sample mean from the true mean	Standard error based on population variance	Standard error based on sample estimate of population variance	Observed deviation of sample mean from the true mean
		Percent of 40.83	*Percent of 40.83*	*Percent of 40.83*	*Percent of 40.83*	*Percent of 40.83*	*Percent of 40.83*
2.5-acre:							
3⅛-percent	1	4.77	4.85	−4.38	4.14	4.36	+9.18
	2		4.95	+1.89		4.26	−.12
	3		4.87	+1.10		4.36	−3.58
	4		4.92	−3.11		4.38	+1.86
6¼-percent	5	3.32	3.11	+4.16	2.88	2.64	−.83
	6		3.16	−.81		2.82	+.37
	7		3.31	−1.03		3.60	+3.87
	8		3.40	−1.47		2.91	+3.08
12½-percent	9	2.27	2.28	+1.86	1.87	1.62	−2.08
	10		2.38	+1.79		1.69	+2.91
	11		2.23	−2.89		1.66	−1.08
	12		2.20	−.42		1.69	−1.40
5-acre:							
6¼-percent	13	4.18	3.99	−3.16	3.50	4.14	+3.80
	14		4.19	+9.58		3.92	−3.92
	15		3.53	+1.08		3.82	−3.70
	16		4.51	−1.49		3.13	−1.10
12½-percent	17	2.86	2.86	+2.47	2.39	2.60	+.86
	18		2.89	+1.74		2.57	+.44
	19		2.77	−5.85		2.30	−.17
	20		2.86	−2.50		2.67	+4.56
10-acre:							
6¼-percent	21	5.18	5.29	−1.10	4.43	3.50	−3.38
	22		5.12	−6.81		4.38	+1.47
	23		5.31	+.51		4.14	+1.05
	24		4.41	+3.23		5.93	+1.79
12½-percent	25	3.54	3.37	+1.59	2.77	2.62	+2.28
	26		3.39	−.39		2.67	−1.69
	27		3.67	+4.58		2.84	+3.43
	28		3.62	+.34		2.94	−4.80

[1] The true mean volume of 2.5-acre plots is 40.83 thousand feet board measure.
[2] Equal numbers of plots selected at random from each block. Blocks are taken here as the smallest square unit of area, sampled equally and with a minimum of 2 plots.

By means of the analysis of variance, however, it may be possible to reduce these standard errors by changing the arrangement of plots to permit the breaking up of total sum of squares into parts contributed from known sources, i. e., into a part due to variation between blocks, and a part due to variation within blocks. Each block is sampled equally with the plots selected at random. To estimate the within mean square, a minimum of two plots to the block is required. For the present, blocks will be considered as the smallest square unit of area so sampled. Degrees of intensity of sampling will be introduced by variations in block and in plot sizes. This arrangement of plots will be contrasted with random selection of plots over the entire area, the latter selection being almost certain to result in an unequal sampling of blocks.

The number of degrees of freedom for the sum of squares due to variation between blocks will be one less than the number of blocks. For the squares due to variation within blocks, the number of degrees of freedom will be the number of sampling units minus the number of block means from which deviations are measured. An application of the F test shows in all cases that the between mean square is significantly the greater, a significant portion of variance irrelevant to sampling error being eliminated by using the within mean square as error variance instead of the total. This analysis is illustrated for quarter-section blocks with 2.5-acre plot size in table 5, using data from table 3 with the necessary additional computations.

TABLE 5.—*Analysis of variance for quarter-section blocks and 2.5-acre plots*

Source of variation	Sum of squares	Degrees of freedom	Mean square	F
Between quarter sections	167,630.7558	35	4,789.4502	22.49
Within quarter sections	482,957.8662	2,268	212.9444	
Total	650,588.6220	2,303	282.4961	

This procedure is legitimate since mean square within blocks, treating each block as a population, is the same within the range of error of random sampling, regardless of differences in block means. The pooled estimates of mean square within all blocks is much more precise than the estimate from a single block, even when applied to a particular block, because of the larger number of degrees of freedom. The reduction in error variance gained by use of blocks of smaller sizes is made clear in table 3.

In making a 6¼-percent cruise with 2.5-acre plots as sampling units, by the random arrangement shown in figure 3, the error variance is 282.4961, and standard error 3.32 percent of the mean. By random within blocks, the 144 plots will average 4 to the quarter section, which is the block unit, as in figure 4 and table 5; error variance will be 212.9444; and the standard error will be reduced to 2.88 percent. All of the reductions in expected standard errors for random within blocks, as compared to random for total population in table 4, are due solely to differences in plot arrangement. For each standard error based on

population variance, four estimates based on actual samples are given and the corresponding deviations of sample means from the true mean.

The effect on sampling error of size, shape, arrangement of plots, and intensity of sampling is reflected in the standard errors as calculated. If the effect of these has been correctly determined—population heterogeneity having been successfully overcome either by randomization alone, or by partial elimination followed by randomization—the deviations of sample means from the true mean

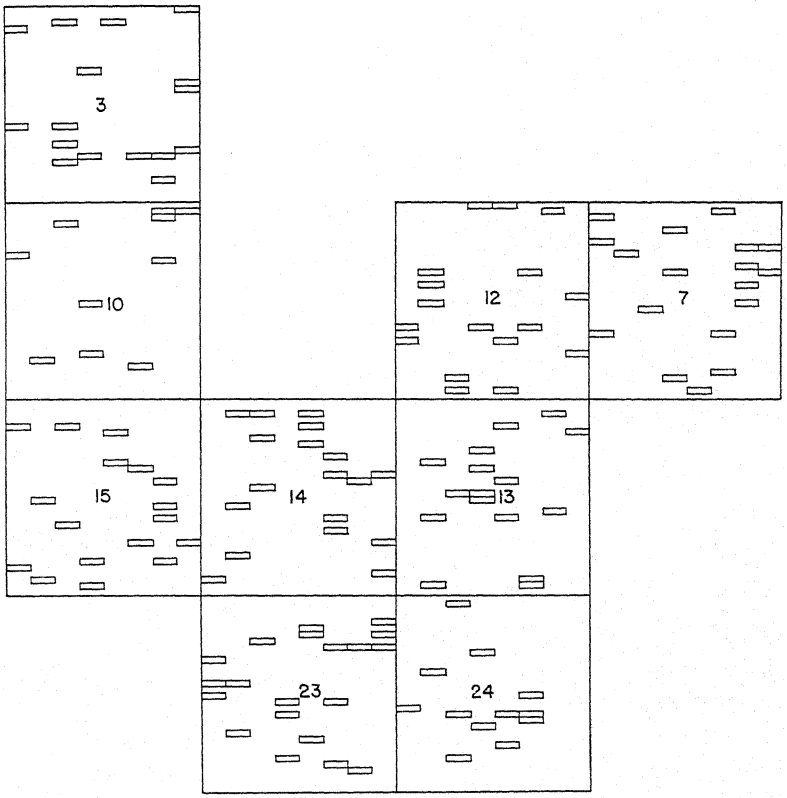


FIGURE 3.—Arrangement of 2.5-acre plots in a 6¼-percent random cruise of nine sections. Plots selected at random from the total population.

expressed in terms of standard units of the normal curve should not differ from a normal frequency distribution by more than may be attributed to error of random sampling alone. If the samples contain the information needed for assessing error due to sampling, the range of deviations in terms of standard units, arrived at from sample estimates of standard error, should likewise agree with expectation of normal grouping. That observed results agree with expected results is shown in table 6. The observed frequencies are within the range expected in 95 percent of such trials.

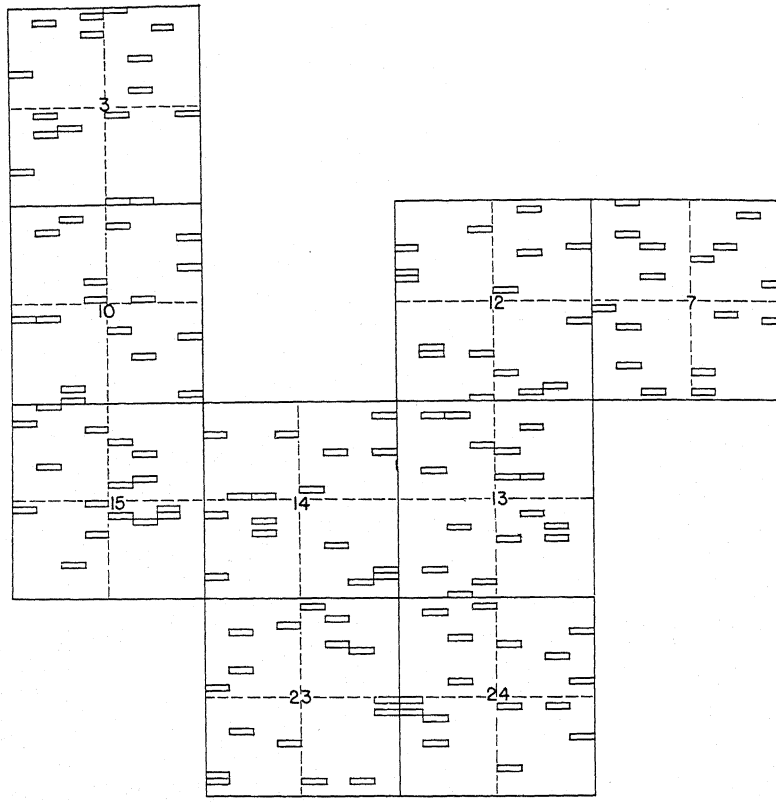


FIGURE 4.—Arrangement of 2.5-acre plots in a 6¼-percent random-within-block cruise of nine sections. Quarter section (40 by 40 chains) taken as the block unit.

TABLE 6.—*Expected and observed grouping of sample means about the true mean*

Range from zero difference in standard units of the normal curve	Expected frequency	Random, observed frequency		Random-within-block, observed frequency	
		Based on true standard error	Based on estimated standard error	Based on true standard error	Based on estimated standard error
Less than 0.253	5.6	7	6	5	4
0.253 to .524	5.6	6	7	6	7
.524 to .842	5.6	6	6	5	4
.842 to 1.282	5.6	5	4	7	8
1.282 and over	5.6	4	5	5	5
Total	28.0	28	28	28	28

STRIP CRUISES

Thus far the most efficient method of sampling indicated is the use of the random-within-block, or "stratified" arrangement of 2.5-acre plots, using blocks of such size that a minimum of two plots is taken

within each. A disadvantage of this arrangement is that it presents some difficulty in locating plots on the ground and in mapping all parts of the area to the desired standard. Within some blocks the plots are clustered together leaving large areas which could not be mapped without running additional line for that purpose alone. The irregularity of line running to locate plots may be partially overcome by use of the strips taken at random within blocks, as shown in figure

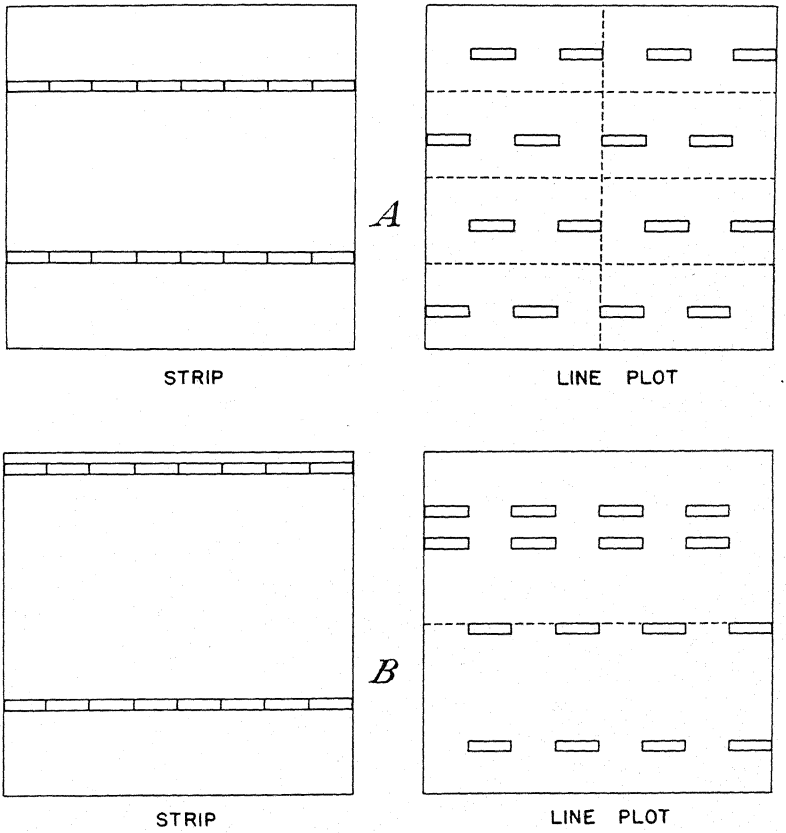


FIGURE 5.—Typical arrangements of plots in 6¼-percent strip and line-plot surveys: *A*, Systematic cruises; *B*, random-within-block cruises. Broken lines indicate block boundaries within sections.

5. Here the sampling unit is the strip, 2.5 by 80 chains, 2 of which are taken from the 32 possible in a section, the block unit. The mean square (in terms of a single 2.5-acre plot) between 28 such cruises is shown in table 7 as 385.5211. According to the previous reasoning, this differs by sampling error alone from mean square between strips within sections, which is estimated here as 425.1970. The F test fails to disprove this hypothesis.

TABLE 7.—*Analysis of variance based on strip cruises of 6¼-percent intensity*

RANDOM-WITHIN-BLOCK STRIP [1]

Source of variation	Sum of squares	Degrees of freedom	Mean square	F [2]	Variance associated with blocks
Between cruises	10,409.0688	27	385.5211	n. s.	
Sections within cruises	277,894.8542	224	1,240.6020	*2.92*	407.70
Strips within sections	107,149.6481	252	425.1970		
Subtotal	385,044.5023	476	808.9170		
Total, between strips	395,453.5711	503	786.1900		
POPULATION VARIANCE [3]					
Between cruises			381.2952		
Sections	109,693.3526	8	13,711.6691	*35.96*	428.49
Strips within sections	106,381.3677	279	381.2952		
Total, between strips	216,074.7203	287	752.8736		
SYSTEMATIC STRIP [4]					
Between cruises	2,835.2736	15	189.0182	2.21	
Sections within cruises	153,411.7346	128	1,198.5292	2.87	390.57
Strips within sections	60,104.5909	144	417.3930		
Subtotal	213,516.3255	272	784.9865		
Total, between strips	216,351.5991	287	753.8383		

[1] Mean volume of 28 cruises=40.79.
[2] n. s.=nonsignificant; values in italics exceed F at 0.01.
[3] True mean=40.83.
[4] Mean volume of 16 cruises=40.83.

True population mean square of strips within sections is 381.2952. Whether or not the estimate obtained differs significantly from this may be tested by the chi-square distribution:

$$\text{Chi square}=\frac{\text{Sum of squares}}{\sigma^2} \qquad (3)$$

For 252 degrees of freedom chi square is 209.5104 at $P=0.975$, and is 297.4360 at $P=0.025$. Substituting 381.2952 for σ^2, solving for each corresponding sum of squares, and dividing by 252 gives the range of mean square expected in 95 percent of such trials. The values obtained are 317.0052 and 450.0433. There is therefore no reason to suspect the sample estimate.

Since variation between sections is significantly greater than variation within sections, the use of total variance for error would give a serious overestimate, and not a valid one.

Systematic strip survey would seem to be quite comparable to strips taken at random within blocks as regards estimates of error mean square. Sixteen 6¼-percent cruises taken side by side include 100 percent of the area or the total population. Population variance of strip cruises is analyzed on this basis in the lower portion of table 7. Variation between such cruises is about one-half that between the random-within-blocks. This advantage in favor of the systematic

cruise is surprisingly large, even considering that such a cruise is more representative in that each half section is sampled equally, whereas in the random the smallest block is the section.

The variation between cruises is less than would be expected in 95 percent of such trials if the sampling were random. The use of the *F* test is not legitimate, however, because the strips are equidistant. The analysis of population variance is made to find how the components of total variance differ in the systematic arrangement as compared to random. Obviously total population variance between strips is the same for both arrangements—the two values shown here are not absolutely the same owing to the use of short-cut methods of computation with the cruise estimates. The component parts do differ. Variation associated with sections is less by systematic than by random, and variation of strips within sections is correspondingly greater. Systematic sample estimates of mean squares do not tend to the same values as do random, and the basis for segregating error mean square is not known. In a single cruise the information for doing this is not provided. With the random cruise, the plot arrangement determines the one and only basis supplied by the data for estimating error mean square, and a single cruise will provide this.

With intensive cruising of fairly uniform stands of timber, such as this, the sacrifice in precision of volume estimate by use of random strip as compared to systematic strip is considerable. In some cases it might be feasible to cruise scattered blocks by random strip until sufficient degrees of freedom are available for a satisfactory estimate of error variance, and then cruise the remainder on the systematic basis; this should give reasonable certainty that the more representative sampling will keep the estimate within the allowable range of error. The advantage gained in mapping is also a consideration favoring this. While for some areas suitable maps are available prior to the cruise, map making in connection with sampling is still an important consideration in most cruising.

LINE-PLOT CRUISES

In the random line-plot arrangement in figure 5, the random sampling unit consists of four 2.5-acre plots spaced equidistant across the section. Two such units are taken at random from the 32 possible in a half section, which is the block unit. The true variance between such cruises is 221.8812 (table 8). In random sampling the appropriate estimate of error is provided by mean square of line plots within half sections, which tends to the true value. The corresponding estimates obtained from 56 such cruises are within a range attributable to random sampling error, and do not differ significantly from each other.

TABLE 8.—*Analysis of variance in line-plot cruises of 6¼-percent intensity*

RANDOM-WITHIN-BLOCK LINE PLOT [1]

Source of variation	Sum of squares	Degrees of freedom	Mean square	F [2]	Variance associated with blocks
Between cruises	10,109.0880	55	183.8016	n. s.	
Half sections within cruises	660,240.1175	952	693.5295	*3.05*	233.18
Line plots within half sections	228,976.3700	1,008	227.1591		
Subtotal	889,216.4875	1,960	453.6819		
Total, between line plots	899,325.5755	2,015	446.3154		
POPULATION VARIANCE [3]					
Between cruises			221.8812		
Half sections	128,774.5115	17	7,574.9713	*34.14*	236.72
Line plots within half sections	123,899.7229	558	221.8812		
Total, between line plots	252,584.2344	575	439.2769		
SYSTEMATIC LINE PLOT [4]					
Between cruises	3,106.6848	15	207.1152	1.21	
Half sections within cruises	177,375.0742	272	652.1142	2.60	200.88
Line plots within half sections	72,102.4804	288	250.3558		
Subtotal	249,477.5546	560	445.4956		
Total, between line plots	252,584.2394	575	439.2769		
Between cruises	3,106.6848	15	207.1152	1.06	
20 by 40 within cruises	394,693.3468	1,136	347.4413	1.58	64.10
Between plots within 20 by 40	252,558.0700	1,152	219.2344		
Subtotal	647,251.4168	2,288	282.8896		
Total, between plots	650,358.1016	2,303	282.3960		
POPULATION VARIANCE [5]					
Between cruises			221.8812	*1.10*	
20 by 40	202,289.9365	71	2,849.1540	*14.18*	89.04
Plots within 20 by 40	448,298.6856	2,232	200.8507		
Total between plots	650,588.6221	2,303	282.4961		

[1] Mean volume of 56 cruises=40.70.
[2] n. s.=not significant; values in italics exceed F at 0.01.
[3] True mean=40.83.
[4] Mean volume of 16 cruises=40.83.
[5] True mean=40.83.

A difficulty arises in connection with the systematic line-plot cruise shown in figure 5, in deciding upon the block unit, i. e., whether the arrangement is comparable to the random line plot or more comparable to random selection of two plots within blocks 20 by 40 chains in dimensions. In practice, with but one cruise supplying the total data, there is no way to determine by which means the most useful information will be obtained. With a population of 16 such cruises, it is possible to determine this for this particular area, but the results will not apply generally. When sampling units are selected independently and at random there is never any doubt as to the one valid basis for estimating error mean square.

If the systematic arrangement permits the same treatment as for the random line plot, the variance associated with blocks is less and mean square within is greater than the true for random sampling. As with the strip survey, such sample estimates tend to the true value for total variance, and variance between cruises is less than that of corresponding random cruises. The apportionment of total variance to sources within cruises is biased. The overestimate from one source is balanced by an underestimate from another source. This is still more pronounced when the 20 by 40 unit is taken as the block. The F test, if legitimate here, would show that both estimates of error mean square are in a range attributable to sampling. Of the two, mean square within 20 by 40's provides the more useful information. In other cases, where a single sample is taken, there would be no assurnace which if either would give a satisfactory estimate of error. The guess with strip survey was as logical as either choice here, and yet was shown to be of little use. In neither case is there any justification for use of total variance as error variance.

RELATIVE EFFICIENCY OF CRUISES

For a standard by which to measure relative efficiency of different cruises we may set up a range of 8.00 percent of the mean at $P=0.05$ for 3⅓-percent cruises. By doubling the intensity to 6¼ percent, we expect a reduction of error to 5.33 percent, and by doubling again to 12½ percent, an error of 4.00 percent. This follows from formula (1). The error mean square necessary to assure this precision is 199.9584 on a single 2.5-acre plot basis. Were the correction to limited population mean made, the efficiency would be greater, since the error mean square would then be reduced by multiplying by the proportion of population not in the sample—as is done by use of formula (2). This correction is not made in table 9 mean squares, since the relative efficiencies would not be changed greatly. As they are given, the effect of decrease of block size with increased intensity of cruise is segregated completely. The reciprocal of 199.9584, or invariance $=0.00500$, is taken as a unit of information per 2.5 acres and the different cruises rated on this basis. One-half unit indicates that sample size would need to be doubled to assure the same precision as that by a cruise supplying a full unit. If costs of cruising were the same per unit of area in the samples, use of the former method would double the cost.

TABLE 9.—*Relative amounts of information by cruises*

Sampling unit and arrangement	Mean square	Invariance [1]	Units of information per 2.5 acres [2]
3⅛-PERCENT SAMPLING			
2.5-acre, 2 plots within quarter section	212.9444	0.00470	0.94
2.5 by 80-chain strips, random	752.8736	.00133	.27
4 2.5-acre plots equidistant along 80-chain line, 2 lines within section	252.0121	.00397	.79
6¼-PERCENT SAMPLING			
2.5-acre, 2 plots within 20 by 40	200.8507	0.00498	1.00
2.5 by 80-chain strips, 2 within section	381.2952	.00262	.52
4 2.5-acre plots equidistant along 80-chain line, 2 lines within half section	221.8812	.00451	.90
5-acre, 2 plots within quarter section	313.2797	.00319	.64
10-acre, 2 plots within half section	477.6623	.00209	.42
12½-PERCENT SAMPLING			
2.5 acre, 2 plots within forty	192.7724	0.00519	1.04
2.5 by 80-chain strips, 2 within half section	323.3341	.00309	.62
4 2.5-acre plots equidistant along 80-chain line, 2 lines within 20 by 80	189.3106	.00528	1.06
5-acre, 2 plots within 20 by 40	291.6305	.00343	.69
10-acre, 2 plots within quarter section	421.6283	.00237	.47

[1] Invariance=$\frac{1}{\text{mean square}}$. Value of 0.00500=1 unit of information.

[2] Based on 1 unit for 8.00-percent error at 0.05 level with 3⅛-percent cruises, 5.66-percent error with 6¼-percent cruises, and 4.00-percent error with 12½-percent cruises. Correction for limited population not included.

The random-within-block 2.5-acre plot cruises meet the standard of precision set up for each of the three different intensities. The random-within-block line-plot cruises are not far behind in the lower intensities and are slightly the more efficient in the 12½-percent intensity. Because of advantages in locating plots on the ground along with satisfactory sampling efficiency, the random-within-block line-plot arrangement of 2.5-acre plots appears to be the best selection.

For a given sampling unit, the increase in information with increased intensity of cruise, as given in table 9, is due to the decrease in block size and the consequent elimination of more variation irrelevant to sampling error.

The 100-percent inventory of the population in this case makes it possible to compare the range of error of systematic cruises with that of similar random cruises. The systematic cruises cannot be treated as were the random in table 9, but for 6¼-percent cruises, both strip and line plot, the true variation between means has been found for this limited population. In both cases the mean squares are less than for similar random cruises. This finding, together with the advantage in mapping, makes it appear doubtful whether these arrangements should be completely discarded simply because single samples do not provide the information needed for estimating sampling error. There are good possibilities that their sampling error can be closely approximated. Where maps are not needed, or are already available, a random cruise can be made in the same time as a comparable systematic cruise. The cruiser must decide whether the

added precision in estimating sampling error is offset by the loss in accuracy of volume estimate.

It has been previously explained[5] that the range of systematic sample means about the true corresponds satisfactorily with the range expected for random-within-block sampling in which square blocks are used. If a number of such blocks in a population were selected at random and cruised by a random arrangement, the pooled estimate of error variance so obtained would then be considered as satisfactorily applicable to the entire population sampled. The remaining blocks could then be cruised by a systematic arrangement, in which the same number, size, and shape of plots would be used. Discarding the restriction as to shape of blocks, blocks 40 by 20 chains (north-south by east-west dimensions) are suitable in the present study for a systematic strip arrangement as shown in figure 5. Random variation within such units tends to 195.5448. This compares very well with the mean square between cruises, 189.0182 in table 7. Obviously, however, this proposed method of approximating error variance could easily be carried beyond a reasonable point by the use of very short strip segments as plots and long, narrow blocks. Probably length of blocks should not exceed twice their width.

Where systematic line plots are taken, some such method of approximating error variance would appear quite safe. The mean square for error of random sampling with 20 by 40 blocks is 200.8507, and the mean square between systematic cruises is 207.1152. The justification for this approach to sampling error is apparent from inspection of the diagrams in figure 5. The systematic arrangements are more representative than the random. For line-plot sampling, each 20 by 20 is sampled by a plot; while in random sampling the plot locations may fall so that some 20 by 20's are not sampled at all and some are sampled with two plots.

F. Yates, chief statistician, Rothamsted Experimental Station, England, has suggested that the sampling error of systematic cruises in which a single sampling unit was taken in the center of each block, might be approximated by using error variance estimates from two randomly selected sampling units in blocks of the same size. Only sufficient random cruising would be done in a population to get an adequate estimate of variance within blocks, and the survey could be continued on the systematic basis. The gain in representativeness by the systematic arrangement as compared to random would then be reflected in reduction of error variance by use of blocks of just half the size needed with random cruises of equal intensity. With the 6¼-percent systematic strip cruise the mean square between the 16 cruises is 189.0182. The mean square between random strips within half sections is shown in the report previously cited as 323.3341. With only 15 degrees of freedom available for estimate, the difference is within a range attributable to error of sampling in an unlimited population. The value 189.0182 is true for the limited population only. The estimates from such cruises in other similar areas might well tend to something like 323.3341. There is no reason, for example, to expect that the strip arrangement should consistently give as

[5] HASEL, A. A. ANALYSIS OF SAMPLING METHODS FOR VOLUME DETERMINATION IN A PONDEROSA PINE FOREST. Calif. Forest and Range Expt. Sta. Prog. Rept. June 1937. [Multilithed.]

close or closer estimates of volume than the line plot, where the spacing apart of plots would appear to sample a block better than by a single strip of contiguous plots. Also the advantage over random strip should not be much greater than can be attributed to more representative sampling alone. It is suggested that this method of approximating error of systematic strip survey be used in preference to the ones previously suggested in which strip segments were considered as plots.

With line-plot arrangements the results by the two methods are quite alike. Variance between line-plot sampling units within 20 by 80's is 189.3106. This differs little from 200.8507, the variance between plots within 20 by 40's, and neither differs significantly from 207.1152, which is based on 15 degrees of freedom.

It has been noted that doubling of intensity, other factors remaining constant, reduces a sampling error of 8.00 percent to one of 5.66 percent. Extremely close estimation of sampling error and small changes in intensity based on estimates of error variance are not important, unless it is realized at the same time that biased error from various sources may contribute much the greater part of total error of cruise estimate. Time saved by lowering intensity can be very profitably spent on reduction of biased error.

PRECISION OF ESTIMATES OF ERROR VARIANCE

The mean squares taken as error variance have been based upon true values for the population dealt with. They are the values to which sample estimates tend. The range within which sample estimates group themselves about the true is dependent solely upon the number of degrees of freedom available for their estimate. The range of 95 percent of such estimates is given for the various random cruises in table 10, derived by use of formula (3). Where the estimate is based on as few as 8 or 9 degrees of freedom a single sample may easily give a very coarse basis for error calculation—not close enough for much reliance. In this respect random plots have a marked advantage over line plots, which in turn are better than strip. By use of a much smaller and probably more efficient size of plot than 2.5 acres, however, the ordinary intensity of cruise in a few sections will give an adequate basis for estimate. Such tests as are given in table 10 should be made the basis for decision as to the number of blocks that should be cruised by a random arrangement before continuing with the systematic as suggested previously.

TABLE 10.—*Range of sample estimates of variance*

Sampling unit and arrangement	Degrees of freedom	Mean square	Range of 95 percent of sample estimates	
				Percent
3⅛-PERCENT SAMPLING				
2.5-acre, 2 plots within quarter section	36	212.9444	123.8059– 319.2763	(−42)-(+ 50)
2.5 by 80-chain strips, random	8	752.8736	203.8405–1648.7932	(−73)-(+119)
4 2.5-acre plots equidistant along 80-chain line, 2 lines within section	9	252.0121	74.4276– 532.1375	(−70)-(+111)
6¼-PERCENT SAMPLING				
2.5-acre, 2 plots within 20 by 40	72	200.8507	139.4796– 270.2647	(−31)-(+ 34)
2.5 by 80-chain strips, 2 within section	9	381.2952	112.6092– 805.1260	(−70)-(+111)
4 2.5-acre plots equidistant along 80-chain line, 2 lines within half section	18	221.8812	100.4136– 388.2921	(−55)-(+ 75)
5-acre, 2 plots within quarter section	36	313.2797	182.1408– 469.7107	(−42)-(+ 50)
10-acre, 2 plots within half section	18	477.6623	216.1724– 835.9090	(−55)-(+ 75)
12½-PERCENT SAMPLING				
2.5-acre, 2 plots within 40	144	192.7724	150.2018– 239.0980	(−22)-(+ 24)
2.5 by 80-chain strips, 2 within half section	18	323.3341	146.3266– 565.8347	(−55)-(+ 75)
4 2.5-acre plots equidistant along 80-chain line, 2 lines within 20 by 70	36	189.3106	110.0652– 283.8334	(−42)-(+ 50)
5-acre, 2 plots within 20 by 40	72	291.6305	202.5212– 392.4172	(−31)-(+ 34)
10-acre, 2 plots within quarter section	36	421.6283	245.1347– 632.1473	(−42)-(+ 50)

APPLICATION OF RESULTS

In cruising it is necessary to make compromises between what is theoretically correct and what is practically possible. Experienced cruisers would never consider using the random arrangement exclusively because it does not lend itself to map making, and the volume estimates are not as close as with the systematic arrangement. It is important, however, to have a valid and adequate estimate of error variance, and this is possible without a great initial sacrifice in time and money. Having that, it is possible to assure an estimate of the required precision so far as sampling error is concerned and by the cheapest method of cruise. Knowing sampling error, it is possible by later checks of estimate against actual cut to segregate error due to biased measurements, and direct efforts toward its reduction which are commensurate with its importance.

A timbered area to be cruised usually consists of several separate populations, which are segregated according to criteria used in mapping. In working out the method of cruise for a population, even though the stand appears uniform to the eye, heterogeneity of variation should be assumed. It follows then that size and shape of plot is an important consideration and tests should be made to find the kind of plot or sampling unit which includes the maximum of stand variation per unit of area. Obviously the minimum size of plot used in the present study is too large to give an indication of what minimum intensity of cruise is possible for a given range of error. As a rough guide until further studies are made, the minimum size with which plot volumes approach the normal type distribution is suggested, although the analysis of variance method does apply to definitely skewed distributions, which are not normal. At any rate the mini-

mum size tested should not often contain zero volume. The time required to cruise these basic plots and multiples of basic plots should be recorded, as well as travel and mapping time per unit of distance between plots. It may prove more economical in practice to use more area in fewer large plots than the theoretical minimum indicated with more and smaller plots. By selecting the plots at random within blocks additional needed information on initial intensity of sampling is gained. Plot arrangement and intensity will require further tests unless previous experience in such stands is available. A more intensive cruise than is believed necessary should be made by use of the random plot or line-plot arrangement within blocks of the minimum size that will be considered. If the stand is not patchy it will be possible to combine adjacent block units into larger blocks, and by pooling within and between sums of squares and degrees of freedom, to estimate variance within blocks of different sizes.

Advantage should be taken of the usual procedure of running cruise lines and orienting plots for the purpose of sampling more intensively in the direction of greatest variation. The Land Office subdivisions are convenient block units, and within sections the cruise lines are generally run north-south or east-west depending on direction of variation. By taking blocks of such size and shape that variation within is kept low, and that between correspondingly high, the maximum of variation irrelevant to sampling error is eliminated. When sufficient degrees of freedom have been built up to provide estimates of variance within a predetermined allowable range, the units of information supplied per basic plot may be calculated for varying arrangements and block sizes. By knowing the average man-hours required to travel to and cruise a plot by each method, the cost per unit of information will give the most efficient method of sampling by a random arrangement. If the cruise is continued with a systematic arrangement of the same intensity of cruise, the estimate of error will be high. A closer approximation can be made by reducing the size of block by a half, so that a single systematically placed sampling unit is in the center of each. Knowing from the preliminary work the variance of random sampling within blocks of this size, this estimate of within variance, when divided by the total number of blocks, or number of systematically placed plots, will give the approximate sampling variance of the mean. By extracting the square root the standard error of the cruise mean is obtained.

The above-suggested procedure for starting a cruise would not need to be repeated in similar stands, except to check on or improve error-variance estimates by cruising randomly selected blocks by the random arrangement. It is intended to apply to intensive cruising of stands which appear uniform within blocks of 40 acres or more, as in the pure ponderosa pine type. A procedure for random sampling within types occurring in small irregular patches is difficult to work out, particularly if a type map is not available beforehand. The same is true of extensive cruising, as in the Forest Survey in parts of the country where types change often. If these scattered small type areas were brought together, however, there is little doubt that analysis of variance would show that they too are heterogeneous populations and that total variance based on systematically spaced plots is not a valid

estimate of error variance. Such an estimate is useful only in that we can be sure that it is an overestimate.

Owing to the volume of timber inventory work in progress or in prospect, as in connection with preparation of timber-management plans and land-use studies, efforts to determine efficient methods of getting adequate samples in major timber types and for various stand conditions would be likely to yield results of considerable practical importance. Existing and proposed experimental forests are expected to represent fairly well the principal stands of timber within national forests. Complete inventories of these areas, with volumes recorded separately by sufficiently small units, would provide a good basis for working out sampling methods for the stands they represent.

CONCLUSIONS

The heterogeneous nature of stand variation within a 5,760-acre tract of the Blacks Mountain Experimental Forest is evidence that timber stands, even though they appear uniform to the eye, are similar to other soil crops in exhibiting systematic and yet partly disordered variation from point to point.

In a heterogeneous population, size and shape of plot are important factors in efficient sampling. A valid estimate of sampling error is possible only when the sampling units are selected independently and at random. By dividing the area into blocks of uniform size and shape, and selecting equal numbers and at least two random sampling units in each, a significant reduction in error variance is possible by Fisher's method of analysis of variance. The customary use of total variance as error variance and the statistical treatment of systematically spaced plots as random sampling units would be legitimate only if the population sampled were shown to be homogeneous. Such a condition seldom if ever exists. It is only by the use of random sampling that the elements contributing to heterogeneity are most likely to be represented in the sample in their true proportion.

Systematic cruises give closer estimates of volume than do similar random cruises and lend themselves better to map making. Since variation within separate blocks in a population varies within a range attributable to sampling error alone, and independently of the block means, only sufficient random cruising is suggested to assure an adequate estimate of error variance. If the remainder of the population is cruised by systematic spacing of sampling units, the estimate of error tends to be a little high. A closer approximation to sampling error is suggested if random variation within blocks of such size that they contain but one systematically placed sampling unit is used for error variance. In this way the gain in added representativeness of systematic sampling, as compared to random, is taken into account.

LITERATURE CITED

(1) CANDY, R. H.
1927. ACCURACY OF METHODS IN ESTIMATING TIMBER. Jour. Forestry 25: 164–169.

(2) FISHER, RONALD AYLMER.
1934. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 5, rev. and enl., 319 pp., illus. Edinburgh and London.

(3) FISHER, R. A., and MACKENZIE, W. A.
1923. STUDIES IN CROP VARIATION. II. THE MANURIAL RESPONSE OF DIFFERENT POTATO VARIETIES. Jour. Agr. Sci. [England] 13: [311]–320, illus.

(4) GOODSPEED, ALLEN.
1934. A MODIFIED PLOT METHOD OF TIMBER CRUISING APPLICABLE IN SOUTHERN NEW ENGLAND. Jour. Forestry 32: 43–46.

(5) IMMER, F. R.
1932. SIZE AND SHAPE OF PLOT IN RELATION TO FIELD EXPERIMENTS WITH SUGAR BEETS. Jour. Agr. Research 44: 649–668, illus.

(6) MUDGETT, BRUCE D., and GEVORKIANTZ, S. R.
1934. RELIABILITY OF FOREST SURVEYS. Jour. Amer. Statis. Assoc. 29: 257–281, illus.

(7) PRESTON, JOHN F.
1934. BETTER CRUISING METHODS. Jour. Forestry 32: 876–878.

(8) ROBERTSON, W. M.
1927. THE LINE-PLOT SYSTEM, ITS USE AND APPLICATION. Jour. Forestry 25: 157–163.

(9) SCHUMACHER, FRANCIS X., and BULL, HENRY.
1932. DETERMINATION OF THE ERRORS OF ESTIMATE OF A FOREST SURVEY, WITH SPECIAL REFERENCE TO THE BOTTOM-LAND HARDWOOD FOREST REGION. Jour. Agr. Research 45: 741–756, illus.

(10) SNEDECOR, GEORGE W.
1937. STATISTICAL METHODS APPLIED TO EXPERIMENTS IN AGRICULTURE AND BIOLOGY. 341 pp., illus. Ames, Iowa.

(11) TIPPETT, L. H. C.
1927. RANDOM SAMPLING NUMBERS. 8 pp., 26 tables. London. (Tracts for Computers No. 15).

(12) WRIGHT, W. G.
1925. VARIATION IN STAND AS FACTOR IN ACCURACY OF ESTIMATE. Jour. Forestry 23: 600–607.

A STUDY OF PROTEIN EXTRACT FROM SOYBEANS WITH REFERENCE TO ITS USE IN FOOD[1]

By SYBIL WOODRUFF, *chief in foods*, EVELYN CHAMBERS, *graduate student*, and HELEN KLAAS, *first assistant in home economics, Illinois Agricultural Experiment Station*

INTRODUCTION

The soybean (*Soja max* (L.) Piper) is unique among plant foods in that it contains about 40 percent of protein. The supply of soybeans gives promise of being abundant, too; Illinois produced in 1937 a little less than 22 million bushels, an increase of about 27 percent over her crop for 1936. Here is a potentially important source of protein, little of which is now being consumed as food. Some people are advocating that the United States extend the food uses of soybeans for the sake of obtaining inexpensive protein. This might be done in either of two ways; by utilizing the beans themselves or by making from the beans a protein concentrate which could take its place with other protein-rich foods.

The purpose of this study was to find a simple method of extracting semipure protein from soybeans and then to determine whether the protein substance could be used advantageously in the preparation of food.

REVIEW OF LITERATURE

The literature relating to soybean proteins and their physical behavior is not extensive and is somewhat confused by the variety of laboratory methods which have been used in extracting the proteins. Moreover, papers bearing on the topic have not been concerned with the uses of the protein in food technology but rather with its chemical nature. There is little in the literature concerning the methods that are being used for removing crude soybean protein for the manufacture of plastics. A recent paper by O'Brien (*10*)[2] gives methods and factory costs for such industrial operations.

Osborne included soybeans in his classic studies of vegetable proteins. In 1898 he and Campbell (*12*) suggested the name "glycinin" for the globulin they dissolved from soybeans with a 10-percent sodium chloride solution. This solvent has continued to be employed more often than any other in extracting globulins. The conventional definition of a globulin would exclude protein extracted by any other solvent than dilute neutral solutions of salts of strong bases with strong acids. Yet O'Hara and Saunders (*11*) have recently reported success in extracting crystalline protein having the characteristics of a globulin with either saturated or normal solutions of sodium chloride. They used orange seed, peanut, and other proteins, but their conclusion that the "ordinary text book definitions of globulins do not adequately consider the solubility properties of globulins" is concurred in by other writers who have worked with still other materials (*4*).

It has been commonly observed that soybean proteins are readily soluble in water. Osborne and Campbell (*12*) said the fact that as

[1] Received for publication March 11, 1938; issued December 1938.

[2] Italic numbers in parentheses refer to Literature Cited, p. 746.

much as 16 percent of the glycinin could be dissolved in water was due to the presence of potassium phosphates in the seed. Both Nakajima (*9*) and Muramatsu (*8*) showed that if soybeans were extracted first with water, there was little remaining protein which would dissolve further in sodium chloride or sodium hydroxide solutions. The last-named author has divided the water-soluble nitrogen of soybeans as follows: Globulins (largely glycinin), 84.25 percent; albumins, 5.36 percent; proteose and nonprotein nitrogen, the remainder.

The usual methods mentioned in the literature for recovering the protein in solid form are dialysis to remove salt and treatment of the extraction liquor with either ammonium sulphate or acid. Hartman and Cheng (*2*, *6*) gave the isoelectric point of soybean protein as pH 5.00. In almost every case reported in the literature the protein product has been dried with alcohol and ether; Hartman and Cheng (*5*) recommended methyl alcohol and ether.

Comments on solubility usually pertain to the ease of dissolving the protein from the seed, not to the solubility of the separated, dried protein. Denatured glycinin was said by Tadokoro and Yoshimura (*13*) to be most soluble in 0.1 to 0.25 normal sodium hydroxide though it was also soluble in several acids. They also found that heating soybeans caused a large percentage of their proteins to be less soluble in water and more soluble in alkaline solutions. Gortner (*3*) believed solubility of globulins to be merely peptization and governed by the ions present. Nevertheless the solubility of glycinin has been variously reported and likewise its ability to coagulate.

METHODS OF EXTRACTION

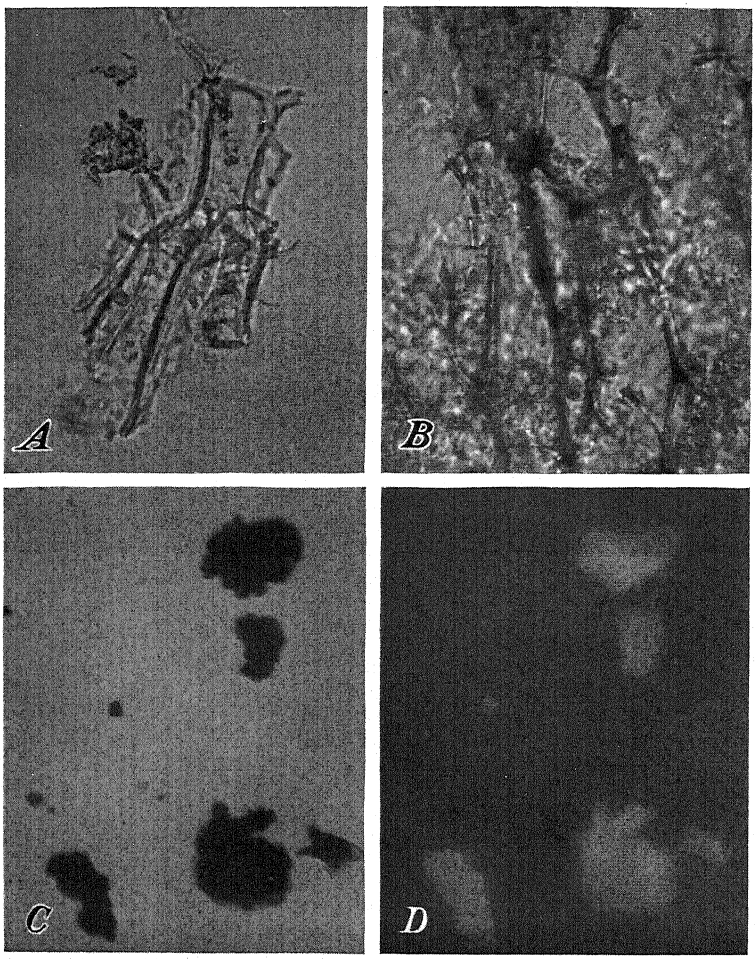
Mature soybeans of two "vegetable" types were used in the experiments, identified throughout the paper by serial Nos. 85666 and 81029. Both had been found in other work (*15*) with soybeans to be satisfactory for food uses. Without preliminary drying, each was ground in a Bauer mill to pass a 60-mesh screen; then extracted continuously for 24 hours with petroleum ether (boiling point 30° to 60° C.) and further ground in a porcelain mortar to pass a 100-mesh sieve. Previous heating of the soybeans was avoided because it tended to make the proteins less soluble.

Commercial fat-free soybean flakes [3] were used for much of the routine experimenting with solvents and methods. They were made from No. 2 Illini soybeans and had been treated only with organic solvent.

SOLVENT

Water was found to be the most practical solvent for protein extraction from the standpoint of yield and purity of product, and time and equipment involved. To 100 g of ground fat-free soybeans was added 750 ml of distilled water, and the suspension was agitated gently in a mechanical shaking machine for 30 minutes at room temperature. It was next poured into a canvas bag and the water "solution" pressed from it in a tincture press. The soybean residue was twice more extracted with fresh portions of 750 ml of water. Suspended matter was separated from the extracts by centrifuging, and the resulting "solutions" became less opaque and viscous in succeeding extractions. Little success was attained in filtering the

[3] Obtained from the Glidden Co., Chicago.



Microscopic appearance of soybean materials: *A*, Fat-free soybean flakes, ground to pass a 60-mesh screen; *B*, residue of flakes after extractions were completed, showing some cells still intact; *C*, soybean protein substance mounted dry showing dense masses of material; *D*, same field as *C* examined between crossed Nicols. Note the luminous character of the protein masses, probably anisotropic. × 900.

extract even with a special filter of acid-alkali-treated asbestos and paper pulp which had been recommended by Hartman and Cheng (*5*). The liquors of the different extractions were kept separate and each in turn was diluted to 800 ml for precipitation of protein.

The commercial flakes absorbed less water than the soybeans, hence only 500 ml of water was used for their first extraction and 400 ml for the second and third. The extracts were diluted to 500 ml before the protein was precipitated.

Solvents other than water were used in a series of preliminary trials with commercial soybean flakes. Solutions of sodium chloride, sodium carbonate, and sodium hydroxide were used in turn. Sodium chloride in 10-percent solution had been frequently used by other investigators; it might well be expected to be the ideal solvent for the principal proteins of soybean, thought to be globulin in nature. Salt solution was not found to be superior to water as a solvent for the purposes of this experiment.

PRECIPITATING AGENT

Dilute acetic acid (about 1 or 2 ml) was added to each water extract of protein, and when the acidity reached pH 5.0 the protein precipitated as a white cloud. Hydrochloric was found to be no more successful than acetic acid. The addition of sufficient calcium chloride to make the liquor 0.02 molar with respect to the salt, aided in precipitating the protein, but this method likewise had no advantage over that of precipitating it with acetic acid. Dialysis through collodion bags effected a satisfactory precipitation of protein from the liquor extracted with sodium chloride solution.

The precipitate settled readily, after which the waste liquor was decanted and centrifuged from it. Successive portions of 70-percent, 95-percent, and then absolute ethyl alcohol were used to wash and dry the protein. It was last washed with ether and worked to dryness on a porous plate. The product was a white, fine powder which showed no tendency to gumminess after it had thus been thoroughly dried. Drying only in air gave a horny substance. No special advantage was found in drying the material with methyl alcohol as recommended by Hartman and Cheng (*5*).

YIELDS

About 52.6 percent of all the nitrogen originally present in the fat-free soybeans was recovered when the protein substance was dissolved in water and precipitated by acetic acid in three successive treatments. A fourth extraction was found not to add significantly to the total yield. The amount of protein left behind in the residue was about 22 percent of the quantity originally present. The fraction that failed to precipitate when acetic acid was added was about 15 percent of the original soybean protein. Unaccountable loss amounted to about 10 percent of the total protein. This waste, thought to be due almost wholly to seepage of the finely ground soybeans through the canvas bag, could be reduced by grinding the beans to pass a 60-mesh screen instead of a 100-mesh. The saving thus effected compensated for the slightly lesser solubility of the protein of the more coarsely ground beans.

Photomicrographs of the original sample, ground to pass a 60-mesh screen, and of the same after it had been extracted three times with water show (pl. 1, *A* and *B*) that it was probably the presence of intact cells which interfered with complete solution of the protein contained

in them and not any inherent insoluble nature of a part of the protein. The photograph also illustrates well the location of the protein and other material in relation to the cells.

ANALYSES

The composition and therefore the purity of the protein substance was found on analysis to vary with different methods of extraction. Nitrogen was determined by the Kjeldahl method; the ashing temperature was 600° C.; samples were dried for moisture determinations at 80° C. in vacuo for 24 hours.

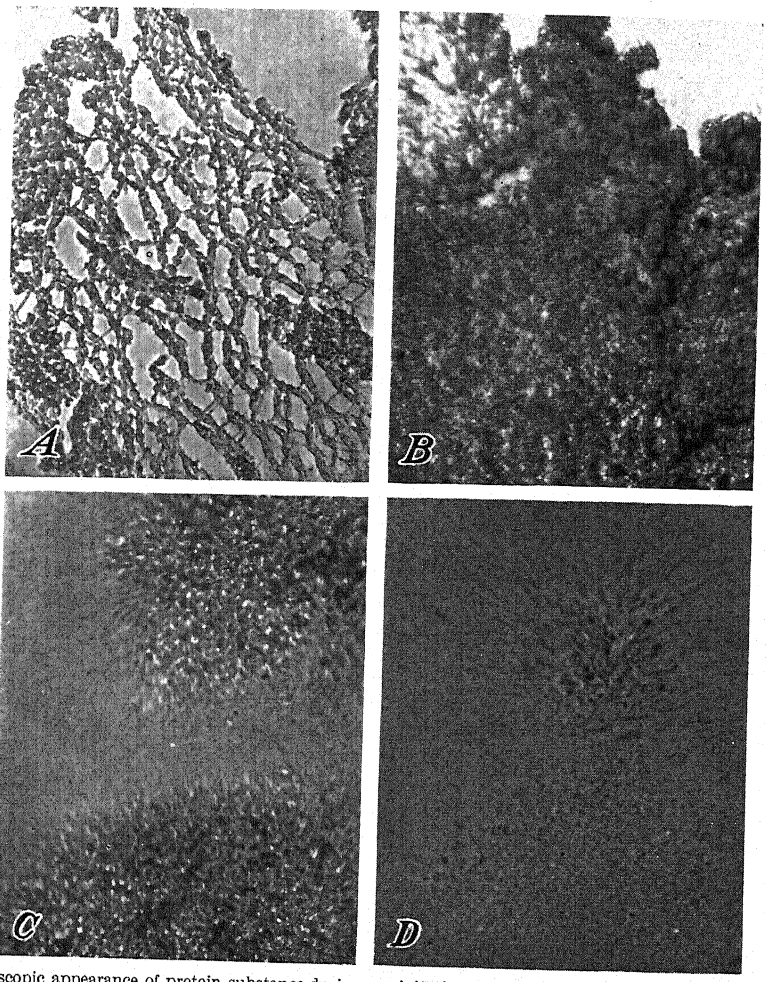
The protein substance yielded by dissolving with water and precipitating with acid, had the highest nitrogen content (16.13 percent) of all samples with the exception of the one dissolved in sodium chloride and dialyzed. The yield by the latter method was small, however. Other authors have reported the nitrogen content of protein substance, obtained by means of various solvents, to be from 12.25 to 17.53 percent. Data in table 1 show that samples obtained with other solvents were less exclusively protein in content; it was largely for this reason that water was selected as the most practicable solvent. The highest yield of protein substance ever obtained was with sodium hydroxide in 0.2-percent solution; but analysis of the material showed it to contain a high percentage of nonprotein constituents, particularly ash.

It is possible that hemicellulose material was dissolved by the alkali treatment just mentioned and was in turn precipitated by the acid used in separating the protein from solution. Occurrence of this kind may even have been observed to some degree when water was used as a solvent. The results given in table 2 show that 39 g of material out of the original 93.6 g of moisture-free beans could be accounted for in neither the protein substance nor the residue of soybeans left after three extractions were made. Of this 39 g of material unaccounted for, 22.78 g were shown by the data to be neither protein nor ash. It is assumed that this unidentified material dissolving out from the soybeans was largely carbohydrate in nature. It seems remarkable that the quantity of water-soluble constituents is as high as this. Further identification of water-soluble carbohydrates is under way.

TABLE 1.—*Analyses of protein substance extracted from soybeans by various methods*

Extraction method		Kind of soybeans	Protein substance		Constituents in moisture-free protein substance			
Solvent	Means of precipitating		Yield [1]	Moisture content	Ash	Nitrogen	Protein	
							N×5.71	N×6.25
			Grams	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Water	Acetic acid	No. 85666 [2]	24.75	5.44	2.34	16.23	92.7	101.4
		No. 81029 [3]	27.37	6.00	2.05	16.13	92.1	100.8
		Commercial flakes. [4]	25.27	6.83	2.24	16.03	91.1	100.2
Sodium chloride solution, 10 percent.	Dialysis	do	10.35	5.78	.57	16.35	93.3	102.2
	Acetic acid	do	13.21	5.63	1.22	13.24	75.6	82.7
Sodium carbonate solution, 10 percent.	do	do	10.36	9.85	4.92	12.55	71.7	78.4
Sodium hydroxide solution, 0.2 percent.	do	do	35.58	4.98	1.21	13.57	77.5	84.8
	Hydrochloric acid.	do	19.83	7.36	1.79	13.00	74.2	81.2
Sodium hydroxide solution, 10 percent.	do	do	10.63	5.92	20.22	13.80	78.8	86.2

[1] From 100 g of fat-free soybeans.
[2] Average of 3 runs.
[3] Average of 2 runs.
[4] Average of 7 runs.



Microscopic appearance of protein substance during precipitation and during peptization: *A*, Threads or chains of newly precipitated protein substance in the mother liquor; *B*, dried protein substance mounted in water, photographed after 10 minutes to show that no peptization had occurred; *C*, dried protein substance mounted in 0.025-percent NaOH solution, after 1 minute standing, shown after peptization had started; *D*, same field as *C* after 10 minutes standing when most of the material had peptized. × 900.

TABLE 2.—*Nonprotein constituents removed from soybeans by extraction with water*

[Amounts based on 100 g of fat-free soybeans No. 85666]

Material	Air-dried weight	Moisture-free weight	Constituents present		
			Ash	Proteins N×5.71	Others by difference
	Grams	*Grams*	*Grams*	*Grams*	*Grams*
Fat-free soybeans	100	93.6	6.32	45.11	42.16
Protein substance	24.7	23.4	.55	21.69	1.16
Residue	34.0	31.2	.79	12.20	18.22
Loss in waste liquor, etc.		39.0	4.98	[1] 11.22	22.78

[1] Analysis of the waste liquor accounted for 6.05 g of this protein loss.

PROTEIN CONVERSION FACTOR

In recording the protein equivalent of the nitrogen percentages found by analysis, the factor 5.71 has been employed, since Jones (7) found it to be the correct one for soybeans. Few investigators even mention the conversion factor they have used, though it appears likely from their results that usually they have used 6.25. That the factor 6.25 was incorrect for the nitrogen yields of this study is indicated in table 1, where the protein content of the substance is shown as computed with each factor. The factor 6.25 made the protein appear to be more than 100 percent of the weight of substance. The protein content of the moisture-free protein substance was 92 percent (N×5.71).

SOYBEAN PROTEIN SUBSTANCE

The general physical behavior of the protein substance was a matter of major concern in the study because the usefulness in food technology of such a concentrated form of protein would be determined by properties like solubility, ease of coagulation, and behavior as a colloid. Several tests designed to reveal its potential functioning in this food capacity were carried out. Samples extracted from the three varieties of soybeans showed no recognizable differences in general behavior.

Separation and identification of the proteins which were precipitated from the water extractions was not one of the purposes of this study. The term "protein substance" has been used by the authors to refer to the recovered material because it was thought unlikely that only an individual protein was present. If other writers are correct, the protein substance was largely the globulin glycinin. It contained, as the above-mentioned analyses indicated, some moisture, a small amount of ash, and about 5 percent of unidentified material.

APPEARANCE AND SOLUBILITY

The protein substance was a tasteless, odorless white powder, the presence of which would be wholly unobjectionable as an addition to foods. So far as could be told, it was not crystalline. During the process of its precipitation from the liquor, microscopic threadlike chains or networks were characteristically present. Such an appearance is shown in plate 2, *A*. During drying the clumps assumed an appearance which would have been described as amorphous except

for the fact that under the microscope they were luminous when examined dry between crossed Nicols. It seemed possible that each clump was made up of innumerable anisotropic units; their luminosity could not be extinguished as the Nicols were turned, but this might have been due to the countless anisotropic units assuming as many different positions. Plate 1, *C* and *D*, shows masses of protein substance mounted dry and examined without and with crossed Nicols.

In water, the clumps exhibited no change in microscopic appearance which could be attributed to dissolving action, though anisotropy did not exist. This is shown in plate 2, *B*, where the clumps remained unchanged after standing in water for 10 minutes. The substance was seen to disintegrate rapidly when it was suspended in a very dilute sodium hydroxide solution (0.025 percent) and watched for several minutes under the microscope. Plate 2, *C* and *D*, shows that the protein clumps were peptizing even after standing 1 minute in the dilute alkali and had almost disappeared after 10 minutes.[4]

But little of the protein substance could be redissolved in water or even in sodium chloride solution. This was true while the material was still moist and also after it had been dried with alcohol. The protein substance was suspended in the solvent and shaken at room temperature for 1.5 hours, at the end of which time only about 12 to 20 percent of it had disappeared. It gave no appearance of dissolving in water though it was peptized readily by either 10-percent sodium carbonate or 0.025-percent sodium hydroxide solutions. The globulin had obviously undergone a transformation to an insoluble protein during the precipitation process. The protein may well have been further denatured during drying, but whether it was greatly exaggerated by alcohol treatment, was not determined.

Both 10-percent sodium carbonate and 6 N acetic acid solutions peptized or softened the protein to a gelatinous mass in a short time. Either of the last two solvents might safely be used in less concentrated solutions for such purpose in food preparation. The dried protein substance, originally extracted from the soybeans with alkaline solutions, dissolved to a somewhat greater extent than the one obtained by the method adopted. But this fact was probably explained by the presence of significant amounts of nonprotein impurities in the protein substance obtained by such solvents.

FOAMING ABILITY

The marked tendency of soybean suspensions to foam was a troublesome property in the processes of extraction, but on the other hand, it was seen to have certain possibilities of practical application. Several tests were made to determine whether the protein substance itself had any foaming ability with the idea in view that it might be put to use in food technology wherever egg white or gelatin are now employed for such purposes.

Eight grams of material, either ground soybeans, or protein substance, or residue from the extractions, in 100 ml of water was whipped at high speed in an electric mixer for 8 minutes. The volume of the foam if one formed at all was measured and then it was emptied into a large funnel containing two layers of cheesecloth. The volume of fluid which had drained from the foam at stated intervals was meas-

[4] Acknowledgment is made of the assistance of Dr. Majel M. MacMasters, associate in home economics, in the preparation of the photomicrographs.

ured as an indication of foam stability, more liquid draining from the less stable foam. The foaming ability of the liquors was measured by treating 100 ml of the liquor as above. The results of the foaming test are given in table 3.

TABLE 3.—*Volume and stability of soybean liquor foams*

Material	pH of solution	Volume of foam [1]	Water drained from foam [1] in—		Remarks
			5 minutes	30 minutes	
		Milliliter	*Milliliter*	*Milliliter*	
Fat-free soybean [2]		600	4	28	Heavy foam, thick consistency.
Residue, moist [2]	6.0	250	70		Thick paste, little foam.
Residue, dried [2]		(3)			
Protein substance:					
Used while still moist [2]		(3)			
Used after dried [2]		(3)			
Protein liquors, first extractions:					
Before precipitation of protein	6.1	1,200	0	60	Stable foam.
After precipitation of protein	5.0	1,600	2	60	Stiff, dry foam.
After precipitation of protein and addition of Na_2CO_3.	6.0	1,400	4	65	Foam like egg white.
After precipitation of protein, followed by removal of heat-coagulable proteins.	5.0	1,700	40	80	Do.
Protein liquors:					
First extraction, after precipitation	5.0	1,600	5	70	Stiff, dry foam.
Second extraction, after precipitation	5.0	1,100	40	85	Stiff foam.
Third extraction, after precipitation	5.2	500	75	85	Thin, watery foam.

[1] Foam produced by 100 ml of solution or suspension.
[2] A suspension contained the equivalent of 8 g of air-dry material per 100 ml.
[3] No foam.

Liquors remaining after most of the protein substance had been precipitated produced fairly stable foams of large volume. One such sample also became ropy and gelatinous after standing overnight in the refrigerator. The presence of mucilaginous constituents has commonly been observed in this laboratory in both green and mature soybeans. In fact, the liquor present in canned green beans, processed at 10 pounds pressure, has usually been found to appear as a soft jelly. No one pH value seemed to be required for the foaming, and the amount of protein dissolved in the liquor did not determine the volume of the foam. With a diminution in amounts of all constituents in succeeding extractions, the volume and stability both decreased.

The soybean residue from the extractions had practically no foaming power in spite of the fact that it still contained about 37 g of protein in 100 g. of moisture-free material. The original fat-free soybeans, on the other hand, formed a fairly stable foam of small volume; part of its stability seemed to be due to suspended matter, for its consistency was very different from that of the other foams.

The results showed that the protein substance, having undergone transformation to an insoluble form during its precipitation, had no ability to produce a foam either when used as freshly precipitated, still moist material or when suspended in water again after it had been dried. Watts (*14*), who investigated the whipping ability of ground, defatted soybeans, had attributed the foams obtained to protein contained by the flour. It is the opinion of the authors that other soybean constituents, e. g., saponins, pectinous or gumlike carbohydrate

derivatives, are responsible to a great degree for the foaming of the ground soybeans and of the liquors. Carbohydrates and like constituents are in process of investigation in the authors' laboratory now. The presence of saponins in soybeans has been reported by several groups of investigators, one of whom, Burrell and Walter (*1*), have recently prepared a crystalline saponin from soybean meal and studied its behavior.

OTHER PHYSICAL PROPERTIES

The protein substance differed from ground fat-free soybeans in several points of behavior. For one thing, it failed to absorb water when moistened and allowed to stand. One of the outstanding characteristics of soybean flour is its ability to absorb and hold large amounts of water. For example, a commercial low-fat soybean flour has been found to take up about 2.5 times its weight of water. This was determined by centrifuging off at high speed all surplus water which the moistened flour failed to retain. By the same test the residue of soybeans left after the proteins were extracted was found to have doubled the original capacity of soybeans to hold water. Milliliters of water absorbed by 100 g of material were as follows:

	Milliliters
Commercial soybean flour, low-fat	245
Commercial soybean flakes, fat-free	255
No. 85666 soybeans, fat-free	225
Residue from No. 85666 soybeans after protein extraction	490

These results indicated that other constituents, probably carbohydrate in nature, were responsible to a large degree for the swelling of soybean products in water.

Seed globulins have usually been reported in the literature to be not heat-coagulable. There is evidence of their slow rate of coagulating in the fact that "soybean milk" can be boiled for several minutes without visible change. Soybean milk is the name given a milky-white, watery suspension which is in common use in the Orient. Tests on the water extracts obtained in these experiments showed that the protein present did not coagulate by heat so long as the pH value was 6.0. After an addition of acetic acid had reduced the pH value to 5.00 and the precipitated protein had been removed, the remaining liquor allowed successive coagula to form at 66°, 80°, and 96° C. as heating and filtering were continued. But in all, the weight of heat-coagulated proteins was only about one-fifteenth of that of the protein known from the analyses to have been present in the liquor.

The very slight solubility of the protein substance has been mentioned already. The small amount which did dissolve, either as freshly precipitated or previously dried protein, showed no visible coagulation even when heated to boiling.

VALUE AS A PROTEIN SUBSTITUTE IN COOKERY

The protein substance being a tasteless white powder was found to have no objectionable features as an article of food. Several tests were made for the purpose of determining whether it had advantages in behavior as a colloid. A few typical dishes were prepared to learn whether it could be satisfactorily substituted for the usual weight of egg protein used for thickening purposes. In a custard no thickening whatever was observed whether the soybean protein was boiled with

the milk or allowed to cook at the lower temperature of the usual baked custard. This was true when both dried and freshly precipitated protein substance were used. Salts or other constituents of the milk, therefore, did not favor peptization of the soybean protein. Muffins were made to contain egg protein, or soybean protein substance in both dried and freshly precipitated forms, or no protein of either kind. The products made with soybean protein were neither better nor worse than those made with no egg at all. The ones containing egg were superior, however, to those without egg. Soybean flour has only slight thickening or binding quality, hence but little of this property has been lost in the conversion of flour to protein substance.

The soybean protein was found to have two advantages over soybean flour; it contained more than twice as much protein and it was free from flavor. Even though it was not found to be a successful substitute for animal proteins for cooking purposes, its presence in a mixture did not interfere with the expected reactions of other components. There is no reason to believe that its digestibility and utilization in the body have been lessened by converting it into a difficultly soluble form of protein during the process of extracting it from the soybean.

It is suggested that soybean protein might be incorporated with any food of moderately thick consistency if there is an advantage in thus increasing the protein content of the diet. Cereals, thickened soups, vegetable dishes, and escalloped foods of many kinds might have the protein substance incorporated in rather large amounts. Further work may reveal ways of reducing the amount of denaturation of the protein as it is precipitated, dried, and stored. Possibly it might then be used still more effectively in food preparation.

SUMMARY

The purpose of the study was to find a simple method of extracting semipure protein from soybeans and then to determine whether the protein substance could be used advantageously in food preparation.

Protein was extracted from two varieties of soybeans and from commercial soybean flakes by treating fat-free finely ground beans with water at room temperature and then precipitating the protein from the extract by acidification with acetic acid to pH 5.0.

The dried protein substance thus obtained represented about 52.6 percent of the nitrogen originally present in the soybeans. It was 92 percent protein ($N \times 5.71$) on a moisture-free basis.

The protein substance was not crystalline but appeared to be anisotropic. Photomicrographs show its luminous appearance between crossed Nicols and also show it in the process of precipitating and peptizing.

It was not measurably soluble in water or salt solution, though acetic acid and sodium carbonate solutions caused it to soften and swell. Suspensions of it did not foam; in this behavior it differed markedly from suspensions of ground fat-free soybeans, the foaming ability of which is probably due partly to nonprotein constituents.

The protein substance did not produce a thickening or binding of food ingredients similar to the effect caused by egg proteins in custards or muffins. Neither did its presence interfere with expected reactions of other components during cooking. It was tasteless and wholly unobjectionable as an addition to other foods.

109365—38——3

It has been suggested that the protein substance might be incorporated in many food dishes for the purpose of adding to their protein content. The substance is thought to offer possibilities as a new source of food protein, and to have the advantage over soybean flour of higher protein content and freedom from flavor.

LITERATURE CITED

(1) BURRELL, R. C., and WALTER, E. D.
1935. A SAPONIN FROM THE SOY BEAN. Jour. Biol. Chem. 108: 55–60, illus.

(2) CHENG, LIBIN T., and HARTMAN, R. J.
1936. STUDIES ON SOY-BEAN PROTEINS. I. A NEW METHOD OF PREPARING SOY-BEAN PROTEINS. Jour. Chinese Chem. Soc. 4: 149–151.

(3) GORTNER, ROSS AIKEN.
1929. OUTLINES OF BIOCHEMISTRY, THE ORGANIC CHEMISTRY AND THE PHYSICO-CHEMICAL REACTIONS OF BIOLOGICALLY IMPORTANT COMPOUNDS AND SYSTEMS. 793 pp., illus. New York and London.

(4) ———, HOFFMAN, WALTER F., and SINCLAIR, WALTON B.
1929. THE PEPTIZATION OF WHEAT FLOUR PROTEINS BY INORGANIC SALT SOLUTIONS. Cereal Chem. 6: 1–17, illus.

(5) HARTMAN, R. J., and CHENG, LIBIN T.
1936. STUDIES ON SOY-BEAN PROTEINS. II. AN IMPROVED METHOD FOR THE PREPARATION OF GLYCININ. Jour. Chinese Chem. Soc. 4: 152–156.

(6) ——— and CHENG, LIBIN T.
1936. ISOELECTRIC POINT OF GLYCININ. Jour. Phys. Chem. 40: 453–459, illus.

(7) JONES, D. BREESE.
1931. FACTORS FOR CONVERTING PERCENTAGES OF NITROGEN IN FOODS AND FEEDS INTO PERCENTAGES OF PROTEINS. U. S. Dept. Agr. Cir. 183, 22 pp.

(8) MURAMATSU, SHUNSUKE.
1920. CHEMICAL AND PHYSIOLOGICAL STUDIES OF THE PROTEINS OF THE SOY BEAN. Jour. Tokyo Chem. Soc. 41: 311–354. [In Japanese. Title also in English.]

(9) NAKAJIMA, KENZO.
1932. STUDIES ON THE PROTEIN AND OIL OF SOY BEAN. Hokkaido Imp. Univ. Faculty Agr. Jour. 31: 165–356, illus.

(10) O'BRIEN, W. J.
1936. SOYA BEAN PROTEINS. Oil and Colour Trades Jour. 90: 1434–1436, 1442.

(11) O'HARA, L. P., and SAUNDERS, FELIX.
1937. STUDIES IN PROTEINS. VI. THE SOLUBILITY OF THE NITROGENOUS CONSTITUENTS OF CERTAIN SEEDS IN SODIUM CHLORIDE SOLUTIONS. Jour. Amer. Chem. Soc. 59: 352–354.

(12) OSBORNE, THOMAS B., and CAMPBELL, GEORGE F.
1898. PROTEIDS OF THE SOY BEAN (GLYCINE HISPIDA). Jour. Amer. Chem. Soc. 20: 419–428.

(13) TADOKORO, T., and YOSHIMURA, K.
1928. CHEMICAL STUDIES ON THE DENATURATION OF PROTEINS. Hokkaido Imp. Univ. Faculty Agr. Jour. 25: 117–132. [Abstract in Chem. Abs. 23: 2996–2997. 1929.]

(14) WATTS, BETTY MONAGHAN.
1937. WHIPPING ABILITY OF SOY-BEAN PROTEINS. Indus. and Engin. Chem. 29: 1009–1011, illus.

(15) WOODRUFF, SYBIL, and KLAAS, HELEN.
1938. A STUDY OF SOY BEAN VARIETIES WITH REFERENCE TO THEIR USE AS FOOD. Ill. Agr. Expt. Sta. Bull. 443, pp. 425–467., illus.

LINKAGE OF THE Q B Gs GROUP IN SORGHUM[1]

By J. C STEPHENS, *associate agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture,* and J. R. QUINBY, *superintendent, Substation No. 12, Texas Agricultural Experiment Station, and agent, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

A rather large number of papers on the genetics of sorghum (*Sorghum vulgare* Pers.) has been published during the past 20 years. The sorghum plant is well adapted to genetic studies because of its growth habits, diversity of characters, and relatively small number of chromosomes. The number of seeds on a single head is usually not less than 500 and may reach a total of 6,000. Most varieties, under suitable conditions, produce tillers and nodal branches and thus continue to bloom intermittently until the plants are killed by frost. So far as known, all varieties of sorghum, including broomcorn and Sudan grass, can be intercrossed freely, and the resulting hybrids are completely fertile.

The inheritance of approximately 50 factor pairs in sorghum has been determined by the writers during the past 12 years. The characters were investigated to determine their linkage relations, and it has not seemed necessary to publish detailed data on simple independent genetic factors. Many of these genetic results were listed, however, in summary tables in an article on sorghum improvement (*10*).[2] This paper reports a linkage group of three factors studied in the coupling phase and the independence of this group from several other factors that have been reported previously by the writers or by other workers. The linked factors are those for reddish and blackish plant color (*Qq*), presence and absence of brown nucellar layer (*Bb*), and normal green and a green-striped chlorophyll deficiency (*Gsgs*).

REVIEW OF LITERATURE

ASSOCIATED CHARACTERS

In 1930 Reed (*23*) reported seedling stem color to be simple in inheritance with red stems dominant to green. Working independently, Karper and Conner (*6*) shortly thereafter reported linkage between seedling stem color and a seedling albino with 41.34 percent crossing over. The factor pairs for this linkage were designated *Rr* and *Ww*, respectively. A two-factor segregation for seedling stem color was reported by Woodworth (*30*).

According to Rangaswami Ayyangar (*13*), Benson and Subba Rao in 1906 observed the association of dull leaf midrib with juicy stalks and white midrib with pithy stalks. Swanson and Parker (*28*) found a higher percentage of smutted plants in rows homozygous for juiciness

[1] Received for publication April 15, 1938; issued December 1938. Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Texas Agricultural Experiment Station at Substation No. 12, Chillicothe, Tex. Technical Series No. 442, Texas Agricultural Experiment Station.

[2] Italic numbers in parentheses refer to Literature Cited, p. 756.

than in rows containing pithy-stalked plants, but they suggested that instead of linkage between smut susceptibility and juiciness there may have been physiological correlation between smut susceptibility and sweetness. These authors found a single-factor difference between dry and juicy stalks and used the symbols *D* and *d* for dominant dry and recessive juicy, respectively. Rangaswami Ayyangar et al. (*18*) used the same symbols in reporting the independent inheritance of juiciness and sweetness, and in a later paper (*17*) they reported a linkage between purple leaf-sheath color and juiciness of stalk with 30 percent crossing over in the F_2 repulsion phase. The factors for purple leaf sheath and its recessive allelomorph, brown leaf sheath, were designated *P* and *p*, respectively.

Rangaswami Ayyangar et al. (*20*, *21*) have observed close association among colors of the anther, stigma, and grain. The associations were assumed to result from expressions of the same color factors. Sieglinger (*25*) found yellow stigmas and colored seed segregating as a unit with white stigmas and white seed, and assumed stigma color to be another expression of the factor for seed color or else due to a very closely linked gene with no crossing over in his populations.

Vinall and Cron (*29*) reported dominance of red glumes over black in the F_1 and a 3:1 segregation in the F_2. Rangaswami Ayyangar et al. (*19*) also found a single-factor segregation and called attention to the association between color of glumes and leaf-sheath color. They designated the factors for dominant reddish purple and recessive blackish purple by the symbols *Q* and *q* and illustrated the phenotypes in color (*19*, *pl. XLII*). Rangaswami Ayyangar et al. (*16*) reported a linkage between the factor pair *Qq* and one of two factors for brown in dry anthers and grain which they designated B_1 and B_2 (*22*). This linkage was studied in the repulsion phase and no crossing over was observed in F_2 and F_3 populations totaling about 10,000 individuals. Recently, Rangaswami Ayyangar (*14*) reported a linkage between the *q* factor and the genes controlling the presence or absence of the nucellar layer, but at this writing has given no data indicating the intensity of the linkage.

Karper and Conner (*6*) reported a lethal yellow seedling character recessive to normal green and designated the factor pair Y_2y_2. Karper (*5*) found starchy endosperm acting as a simple dominant to waxy with the F_2 seeds on F_1 heads segregating 3:1 and F_1 pollen segregating 1:1. The symbols *Wx* and *wx* were used for the factor pair. Karper et al. (*7*) reported a linkage of the factor pairs Y_2y_2 and *Wxwx* with 26.5 percent crossing over.

MISCELLANEOUS INDEPENDENT CHARACTERS

The symbols *A* and *a* have been used to designate the pair of factors for awnless and awned, respectively. Vinall and Cron (*29*) found a segregation of 3 awnless to 1 awned in the F_2 of a Dwarf milo × feterita cross, but Sieglinger et al. (*26*) pointed out that the segregation in this particular cross is 1 tip-awned : 2 weak-awned : 1 strong-awned. Ramanathan (*12*) reported 3 short-awned to 1 long-awned, and Sieglinger (*25*) found 3 awnless to 1 awned in a Blackhull kafir × darso cross. In this cross Sieglinger observed independent inheritance of awns and seed color.

Karper and Conner (*6*) reported a virescent yellow seedling which occurred in F_2 populations in the ratio of 3 green to 1 virescent yellow. The factor pair concerned was designated V_2v_2.

Karper and Stephens (*8*) reported normal flowers dominant to antherless with a 3:1 segregation in the F_2, and Martin (*10*) used the symbols *Al* and *al*, respectively, to designate the factor pair involved. Karper and Stephens also gave the inheritance of twin-seeded spikelets, resulting from full development of the lower normally sterile flowers, as simple dominant to single-seeded spikelets. No symbols were used for the factor pair.

SEED COLOR CHARACTERS

Seed colors have been studied more intensively in India than in America, and several factors have been reported by workers in India which have not been mentioned in papers published in this country. Graham (*4*) found red (*R*), yellow (*Y*), and white an allelomorphic series except that some whites were undeveloped reds requiring the presence of yellow for expression of color. Graham's hypothesis was revised by Rangaswami Ayyangar et al. (*20, p. 603*). In this revision the authors assumed the following factors for color of sorghum grains:

Red	*YYRRWWII*
Pink	*YYRRWWii*
Yellow	*YYrrWWII*, *YYrrWWii*
White (red base)	*YYRRwwII*, *YYRRwwii*
White (yellow base)	*YYrrwwII*, *YYrrwwii*

A basic yellow plant color is represented by the symbols *YY* but apparently no allelomorph has been found. A factor pair for red grains was designated *Rr*. The factor *R* masks yellow and when *R* is present the grains are red, pink, or white depending on additional factors for intensity of color and wholeness of color. The allelomorph, *r*, permits expression of yellow. Red-grained plants have a color-intensity factor assigned the symbol *I*, but potentially red-grained plants are pink-grained when only the allelomorph, *i*, is present. A factor pair for wholeness of color represented by the symbols *Ww* determines whether the seeds are colored or white. When the factor *W* is present the seeds are red, pink, or yellow; but when only the allelomorph, *w*, is present the seeds are white except for a slight expression of color at the base of grains. This color at the base of grains is red or yellow as determined by the factor pair for red color, *Rr*.

The seed color of Yellow milo, as the name implies, has been considered yellow in America. According to Rangaswami Ayyangar and associates (*15*), the color of Yellow milo is pink rather than yellow. These authors gave Yellow milo seed color the factorial composition *YYWWRRii*. Since they consider the seed color of Yellow milo not true yellow, probably no varieties grown in the United States would be regarded by them as yellow-seeded. Many of the sorghums imported from India will not mature in this country in field plantings, probably primarily because of length-of-day reaction. Yellow-grained types distinctly different from Yellow milo in appearance have been planted at Chillicothe but have not matured.

Vinall and Cron (*29*), Sieglinger (*24*), and Swanson (*27*) have used the symbol *R* to designate a factor for red seed color. Conner and Karper (*2*) reported seed color inheritance in White milo × Yellow milo, Blackhull kafir × Red kafir, and Blackhull kafir × Pink kafir crosses without assigning symbols. Under the hypothesis of Rangaswami Ayyangar and associates these American studies with segregation of red and white or yellow and white seed colors involve the factors *Ww* for wholeness of color and not factors for red or yellow.

Vinall and Cron (*29*) found a 9 brown-seeded to 7 white-seeded segregation in the F_2 generations of feterita × Blackhull kafir and reciprocal crosses. Both feterita and Blackhull kafir are white-seeded varieties. These authors assumed a factor *B* for brown specks carried by kafir and a factor *S* carried by feterita for a spreader which diffused the brown specks, giving the whole seed a brownish color in F_1; the factors were inherited independently. Sieglinger (*24*) revised the hypothesis and assigned the symbol *B* to brown nucellar layer carried by feterita. The dominant smooth or glossy pericarp of kafir was designated *S*, and with both *B* and *S* present the epidermis was brown. Swanson (*27*) assumed the factor *S* to determine the development of a vestigial mesocarp while the allelomorph, *s*, determined a well-developed starchy mesocarp. The thick mesocarp, *ss*, was said to mask nucellar color and inhibit even slight expression of color in the pericarp from the factor *B*. Regardless of the exact functions of the factor pair *Ss*, it was agreed by these investigators that in the presence of both *B* and *S* the color of seed was buff or brown and *B* and *S* were inherited independently.

The brown washes of Rangaswami Ayyangar et al. (*22*), designated B_1 and B_2, may be assumed to be distinct from nucellar layer, since at the time of their report these workers stated (*p. 88*) that in their experience "the nucellar brown has not been met with," and one of the brown washes was reported to be very closely or absolutely linked with the plant color factors *Qq* (*16*). Later Rangaswami Ayyangar (*14*) reported nucellar layer linked with the blackish-purple plant color gene *q* and therefore linked with brown wash. It may be suggested that his statement (*14, p. 200*) that "almost all the varieties with brown nucellus are (varieties) whose leaf sheaths and glumes are blackish-purple" should be qualified to except broomcorn and the kaoliangs from China, Chosen, and Manchuria. Among the many importations of kaoliangs into America a large majority carry the red plant color and have nucellar layer present.

It may be expected, of course, that with further studies and exchange of materials the different views will be harmonized and the mounting number of distinct factors reported will be recognized by all workers.

DESCRIPTION OF CHARACTERS

PLANT COLOR

The influence of one of the pairs of genes affecting plant color in sorghum is expressed in injured tissue throughout the plant and in mature glumes. In some varieties the injured or decaying seminal and coronary roots of seedlings or older plants, the tissues injured by aphids, chinch bugs, or other insects, particularly in leaf sheaths, the tissues surrounding areas attacked by foliage diseases, and the small spots on seed regularly turn a reddish color; and in these varieties the

mature glumes are some shade of red, usually dark but distinctly of a reddish cast. In other varieties the color is much darker and is definitely blackish. Distinction between the two colors is made without difficulty in good seasons although seasonal conditions and modifiers influence development of color, particularly in glumes.

Vinall and Cron (*29*) reported red glumes dominant to black and a 3:1 segregation in the F_2. Rangaswami Ayyangar et al. (*19*) associated glume color with leaf-sheath color. They designated the dominant factor for red *Q* and the allelomorph for black *q*.[3]

BROWN NUCELLAR LAYER

A brown nucellar layer is present in kernels of some varieties of sorghum and absent in others. This structure is a single layer of thick brown cells (fig. 1). The presence of a nucellar layer can be

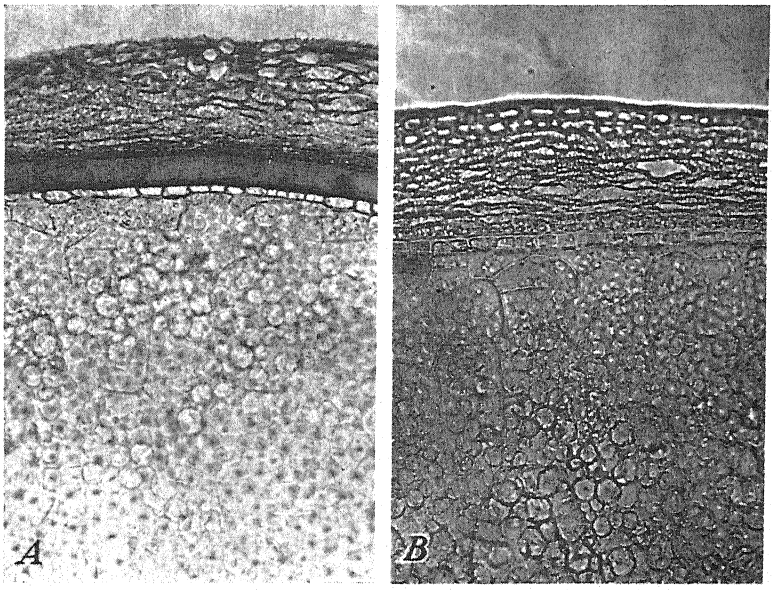


FIGURE 1.—Cross sections of mature sorghum seed showing presence and absence of brown nucellar layer: *A*, Brown hegari (*BBSSrr*); *B*, Dwarf Yellow milo (*bbSSRR*). ×160.

determined readily by scraping off outer layers of the seed coat. Since the symbols *Bb* appear to have been used by Sieglinger (*24*) for brown nucellar layer previous to their use by Rangaswami Ayyangar et al. (*22*) for brown wash, they are used in this paper to represent the factor pair for presence and absence of nucellar layer.

GREEN STRIPED

Chlorophyll-deficient types occur frequently in sorghum. This particular chlorophyll deficiency was observed in a plot of (Blackhull kafir×feterita)×Blackhull kafir, F. C.[4] 8951, at Chillicothe in 1932.

[3] In some American varieties the red color of plant and glume is recessive to black, but the interrelationships have not been completely worked out.

[4] F. C. denotes accession number of the Division of Forage Crops and Diseases.

The character was later found to be monofactorial and recessive to normal green. The typical recessive condition can be best described as similar in appearance to the green-striped in corn illustrated by Lindstrom (*9, pl. III*). The dilution of chlorophyll varies from plant to plant and at different stages of growth. Seedlings sometimes emerge yellow but usually are green and turn yellow or striped within 2 or 3 weeks. In some cases no fading of green occurs until plants are 10 or 12 inches tall. Later most of the plants turn green and at maturity cannot be distinguished from normal greens except that frequently short streaks in the leaves fail to develop color. Some plants remain striped to maturity. The character is sometimes lethal, but green-striped seedlings usually survive unless environmental conditions, such as dry weather or chinch-bug infestation, are severe.

Continued selection of extreme types through three generations failed to produce lines that varied from each other or from parent stocks. The character is influenced appreciably by environment and frequently appears on one end of a series of homozygous rows before it can be detected at the other end. In separating phenotypes it has been necessary to stake the recessive plants intermittently over a period of 3 or 4 weeks, but this separation has been reasonably accurate as determined by the F_3 progenies.

LINKAGE OF Q, B, AND Gs

Linkage of glume color and nucellar layer was observed at Chillicothe in 1929 and briefly mentioned in the Texas Station Annual Report of 1930 (*11*). Rangaswami Ayyangar (*14*) has reported the same linkage but has not given the intensity. The linkage between nucellar layer and green-striped was found in 1935. Data accumulated over a period of years for the coupling phase of the two linkages are shown in table 1. Cross-over values of approximately 16 per-

TABLE 1.—*Segregation in the F_2 coupling phase of plant color (Qq) with nucellar layer (Bb) and nucellar layer with green-striped (Gsgs)*

Years	Factors	Total population	Classes				Cross-over	Standard error
			AB	*Ab*	*aB*	*ab*		
							Percent	
1929–36	*Qq* and *Bb*	8,922	6,167	656	649	1,450	16	0.4064
1935–36	*Bb* and *Gsgs*	2,093	1,466	113	121	393	12	.3503

cent between glume color and nucellar layer and 12 percent between nucellar layer and green-striped are indicated.

An F_2 population of Brown kaoliang, S. P. I.[5] 66384 × green-striped F. C. 8951, in which the three characters were brought together in the coupling phase, was grown in 1936. The single-factor inheritance of each character in this particular population is shown in table 2.

The parental combinations and recombinations of the three characters in the F_2 generation are given in table 3. The indicated order of genes and cross-over percentages is *Q* (13.2) *B* (11.3) *Gs*.

[5] S. P. I. denotes accession number of the Division of Plant Exploration and Introduction, formerly Office of Foreign Seed and Plant Introduction.

TABLE 2.—*Classes of plant color (Qq), nucellar layer (Bb), and green-striped (Gsgs) in an F_2 population of Brown kaoliang, S. P. I. 66384, × green-striped ((Blackhull kafir × feterita) × Blackhull kafir), F. C. 8951, grown in 1936, showing single-factor segregation for each of the characters*

Factors	Total population	Classes			χ^2	*P* value between—
		Dominant	Recessive			
		Number	*Number*	*Percent*		
Plant color, *Qq*	1,960	1,470	490	25.00		
Nucellar layer, *Bb*	1,960	1,476	484	24.69	0.097	0.80–0.70
Green-striped, *Gsgs*	1,960	1,484	476	24.29	.533	.50–.30

TABLE 3.—*Parental combinations and recombinations of plant color (Qq), nucellar layer (Bb), and green-striped (Gsgs) in an F_2 population coupling phase of Brown kaoliang, S. P. I. 66384 × green-striped ((Blackhull kafir × feterita) × Blackhull kafir), F. C. 8951, grown in 1936*

Combinations and recombinations	Factors	Population		Percent
		Number	Total	
Parental combinations	*QBGs*	1,246	1,514	77.245
	qbgs	268		
Recombinations between *Qq* and *Bb*	*qBGs*	123	224	11.429
	Qbgs	101		
Recombinations between *Bb* and *Gsgs*	*QBgs*	98	188	9.592
	qbGs	90		
Double recombinations	*QbGs*	25	34	1.735
	qBgs	9		
Total			1,960	
Recombinations and double recombinations.	*Qq–Bb*			11.429+1.735=13.164
	Bb–gsgs			9.592+1.735=11.327
	Qq–Gsgs			
Total				24.491

INDEPENDENT INHERITANCE OF Q B Gs GROUP WITH SOME OTHER REPORTED SINGLE-FACTOR CHARACTERS

The segregation in F_2 of one or more members of the linkage group with certain other characters is shown in table 4. The segregations indicate independent inheritance or cross-over values so high that linkage cannot be detected. The inheritance of each of these characters has been reported as simple 3:1, although a two-factor segregation has also been reported for seedling stem color.

In the counts accumulated at Chillicothe during the past several years, the segregations for some of the characters deviate significantly from a 3:1 ratio. This condition is frequently found in inheritance studies, and various causes may be responsible. In sorghum some phenotypes are hard to classify at times because drought or chinch-bug damage retards normal development of characters, and no doubt a few errors in classification have been made. In other cases it is likely that there may have been modifications in gametic ratios or differential germination or emergence of sporophytes. Regardless of the cause of these deficiencies in the recessive classes, there is no reason to assume more than a single-factor difference in any specific case, and

we are here concerned primarily with the relation of these factors with the *Q B Gs* linkage group. Collins' formula (*1*) was used to calculate expected classes for computation of χ^2. In using Fisher's table of χ^2 (*3*) to obtain a *P* value, *n* equals 2 because two entries in the four frequency classes can be made arbitrarily, leaving only 2 degrees of freedom.

TABLE 4.—*Independent segregation in the F_2 generation of miscellaneous characters with members of the linkage group Q (plant color), B (nucellar layer), and Gs (green-striped)*

Independent characters	Factors (segregating pairs)	Population								χ^2	*P* value [1] between—
		Total	Observed F_2				Calculated F_2				
			AB	*Ab*	*aB*	*ab*	*A'B'*	*A'b'*	*a'B'*	*a'b'*	
		Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	
Spreader	*Qq-Ss*	618	384	100	95	39	375	109	104	30	4.438	0.20-0.10
Pericarp color	*Bb-Rr* [2]	3,319	2,485	626	208	------	2,490	622	207	------	.041	.98-.95
	Bb-Rr	6,773	3,943	1,214	1,222	394	3,933	1,224	1,232	384	.448	.80-.70
	Rr-Qq	139	75	30	25	9	76	29	24	10	.189	.95-.90
	Rr-Qq	5,713	3,359	990	1,019	345	3,333	1,016	1,045	319	3.634	.20-.10
Awns	*Qq-Aa*	6,711	3,908	1,317	1,226	359	3,846	1,280	1,189	396	6.034	.05-.02
	Bb-Aa	10,402	5,929	2,024	1,847	602	5,945	2,008	1,831	618	.724	.70-.50
Juiciness	*Dd-Qq*	2,773	1,577	546	485	165	1,579	544	483	167	.042	.98-.95
	Dd-Bb	8,251	4,718	1,470	1,567	496	4,714	1,474	1,571	492	.057	.98-.95
	Dd-Gsgs	1,760	1,008	319	331	102	1,010	317	329	104	.067	.98-.95
Seedling stem color	*Rsrs-Qq*	3,023	1,786	478	572	187	1,766	498	592	167	4.101	.20-.10
	Rsrs-Bb	3,762	2,197	620	694	251	2,165	652	726	219	8.130	.02-.01
	Rsrs-Gsgs	1,148	681	185	214	68	675	191	220	62	.986	.70-.50
Antherless	*Alal-Bb*	428	241	97	71	19	246	92	66	24	1.795	.50-.30
	Alal-Gsgs	133	75	22	28	8	75	22	28	8	------	------
Waxy	*Qq-Wxwx*	1,971	1,232	322	347	70	1,245	309	334	83	3.225	.20-.10
	Bb-Wxwx	1,971	1,224	309	355	83	1,228	305	351	87	.295	.90-.80
Virescent yellow	$V_{2}v_{2}$-*Bb*	1,029	630	185	161	53	626	189	165	49	.535	.80-.70
Twin-seeded	*Bb-Tsts*	188	100	37	35	16	98	39	37	14	.538	.80-.70

[1] $n=2$.
[2] Ratio expected, 12:3:1.

Pericarp color—this term being used to exclude the brown resulting from the presence of both *B* and *S* (*27*)—was found to be independent of *B* by Vinall and Cron (*29*), Sieglinger (*24*, *25*), and Swanson (*27*). In table 4, independent segregation of *Bb* and *Rr* is shown in two groups of data. The first group contains the factors *SS*, and all phenotypes having nucellar layer present (*B*) are brown. A 12 brown : 3 red : 1 white segregation is expected. In the second group phenotypes *R* and *r* were determined from stigma color, and the expected segregation is 9 *BR* : 3 *Br* : 3 *bR* : 1 *br*.

Likewise two groups of data are given with *Rr* and *Qq* segregating. The first group shows counts among the homozygous *ss* plants of a larger homozygous *BB* population with 9 *QR* : 3 *Qr* : 3 *qR* : 1 *qr* expected. In the second group *R* and *r* were determined from stigma color. The *R* factor appears to segregate independently with both *B* and *Q*.

As previously mentioned, on the hypothesis of Rangaswami Ayyangar et al. (*20*) these segregations involve the *Ww* (wholeness of color) factors rather than the *Rr* factors. The separations at Chillicothe were made on the basis of presence or absence of color on exposed portions of the grain or in stigmas, and no critical study of seed color factors has been made.

The segregation of awnless and awned lemmas apparently is independent of *Qq* and *Bb*. The *P* value for the observed segregation of *Qq* with *Aa* is low and on the border line of significant deviation from the expected, but the segregation of *Bb* with *Aa* is well within expectation, and there is no indication of linkage. Sieglinger (*25*) found no association in inheritance of awns and nucellar layer.

The symbols *Rr*, designated by Karper and Conner (*6*) to represent red seedling stem and the recessive allelomorph, green seedling stem, were used in several earlier papers to represent the factors for red and white seed. It is suggested, therefore, that *Rsrs* be used as symbols for red and green seedling stems. In the counts recorded in table 4 the segregation of *Rsrs* with *Bb* deviates significantly from the expected for independent inheritance. On the other hand, no significant deviation occurs in the segregation of *Rsrs* with either *Qq* or *Gsgs*, and in the chromosome group the latter two pairs are located on opposite sides from *Bb*. Considering all of the data, there seems to be no indication of linkage between *Rsrs* and the *Q B Gs* group.

Segregation of the *Qq* and *Bb* factor pairs with starchy and waxy endosperm indicates independent inheritance. Tabulations of glume color with endosperm and nucellar layer with endosperm were made from the same F_2 populations. *Wx* and *wx* were determined from pollen tests.

The F_2 population was small, but the available data indicate independent inheritance of the factors for twin-seeded spikelets and nucellar layer. Since no symbols for the factors for twin-seeded and single-seeded spikelets have been given previously, *Ts* and *ts*, respectively, are suggested.

SUMMARY

Other workers have reported three linkages in sorghum in which cross-over percentages have been determined. The phenotypes, factor symbols, and cross-over percentages are as follows: Red or green seedling stems (*Rsrs*) and green or albino seedlings (*Ww*), 41.34 percent; starchy or waxy endosperm (*Wxwx*) and green or lethal yellow seedlings (Y_2y_2), 26.5 percent; and purple or brown leaf sheaths (*Pp*) and dry or juicy stalks (*Dd*), 30 percent.

This paper reports a sorghum linkage group of three pairs of genes studied in the coupling phase. The contrasting phenotypes are reddish and blackish plant color, conspicuously shown in glumes; presence and absence of nucellar layer; and normal green and green-striped plants. The factor pairs for the first two characters have been designated *Qq* and *Bb*, respectively, by other authors, and symbols *Gsgs* are suggested for the third. Each of these factor pairs shows complete dominance in the F_1 and simple 3:1 segregation in the F_2. The indicated order of genes and cross-over percentages is *Q* (13.2) *B* (11.3) *Gs*.

Independent inheritance, or cross-over values so high that linkage could not be demonstrated, was found for one or more members of the linkage group with each of the following pairs: Presence and absence of spreader (*Ss*); colored and white seed (*Rr*); awnless and awned lemmas (*Aa*); dry and juicy stalks (*Dd*); red and green seedling stems (*Rsrs*); normal and antherless flowers (*Alal*); starchy and waxy endosperms (*Wxwx*); green and virescent yellow seedlings (V_2v_2); and twin-seeded and single-seeded spikelets (*Tsts*).

LITERATURE CITED

(1) COLLINS, G. N.
1924. MEASUREMENT OF LINKAGE VALUES. Jour. Agr. Research 27: 881–891.
(2) CONNER, A. B., and KARPER, R. E.
1923. THE INHERITANCE OF SEED COAT COLOR IN CERTAIN CROSSES IN GRAIN SORGHUM. Jour. Amer. Soc. Agron. 15: 338–344.
(3) FISHER, R. A.
1928. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 2, rev. and enl., 269 pp., illus. Edinburgh and London.
(4) GRAHAM, R. J. DOUGLAS.
1916. POLLINATION AND CROSS-FERTILIZATION IN THE JUAR PLANT (ANDROPOGON SORGHUM BROT.). India Dept. Agr. Mem., Bot. Ser. 8: [201]–216, illus.
(5) KARPER, R. E.
1933. INHERITANCE OF WAXY ENDOSPERM IN SORGHUM. Jour. Heredity 24: 257–262, illus.
(6) ——— and CONNER, A. B.
1931. INHERITANCE OF CHLOROPHYLL CHARACTERS IN SORGHUM. Genetics 16: 291–308.
(7) ——— QUINBY, J. R., and JONES, D. L.
1934. INHERITANCE AND IMPROVEMENT IN SORGHUM. Tex. Agr. Expt. Sta. Ann. Rept. 47: 65–66.
(8) ——— and STEPHENS, J. C.
1936. FLORAL ABNORMALITIES IN SORGHUM. Jour. Heredity 27: 183–194, illus.
(9) LINDSTROM, E. W.
1918. CHLOROPHYLL INHERITANCE IN MAIZE. N. Y. (Cornell) Agr. Expt. Sta., Mem. 13, 68 pp., illus.
(10) MARTIN, JOHN H.
1936. SORGHUM IMPROVEMENT. U. S. Dept. Agr. Yearbook 1936: 523–560, illus.
(11) QUINBY, J. R.
1930. SORGHUM GENETICS. Tex. Agr. Expt. Sta. Ann. Rept. 43: 135.
(12) RAMANATHAN, V.
1924. SOME OBSERVATIONS ON MENDELIAN CHARACTERS IN SORGHUM. Jour. Madras Agr. Students' Union 12: 1–17, illus.
(13) RANGASWAMI AYYANGAR, G. N.
1935. JUICINESS AND SWEETNESS IN SORGHUM STALKS. Madras Agr. Jour. 23: 350–352.
(14) ———
1936. LINKAGE BETWEEN THE BLACKISH PURPLE OF SHEATH AND GLUME, AND NUCELLAR BROWN IN SORGHUM. Current Sci. 5: 200–201.
(15) ——— PANDURANGA RAO, V., and KUNHIKORAN NAMBIAR, A.
1935. THE INHERITANCE OF SOME CHARACTERS IN CROSSES WITH THE SORGHUMS, MILO AND KAFIR. Indian Acad. Sci. Proc. 2: 508–522, illus.
(16) ——— SANKARA AYYAR, M. A., and PANDURANGA RAO, V.
1934. INHERITANCE OF CHARACTERS IN SORGHUM—THE GREAT MILLET. V. LINKAGE BETWEEN SHEATH, GLUME AND DRY ANTHER, GRAIN COLOURS. Indian Jour. Agr. Sci. 4: 90–95, illus.
(17) ——— SANKARA AYYAR, M. A., and PANDURANGA RAO, V.
1937. LINKAGE BETWEEN PURPLE LEAF-SHEATH COLOUR AND JUICINESS OF STALK IN SORGHUM. Indian Acad. Sci. Proc. 5 (B): 1–3, illus.
(18) ——— SANKARA AYYAR, M. A., PANDURANGA RAO, V., and KUNHIKORAN NAMBIAR, A.
1936. MENDELIAN SEGREGATIONS FOR JUICINESS AND SWEETNESS IN SORGHUM STALKS. Madras Agr. Jour. 24: 247–248.
(19) ——— VIJIARAGHAVAN, C., GOMATHINAYAGAM PILLAI, V., and SANKARA AYYAR, M. A.
1933. INHERITANCE OF CHARACTERS IN SORGHUM—THE GREAT MILLET. II. PURPLE PIGMENTATION ON LEAF-SHEATH AND GLUME. Indian Jour. Agr. Sci. 3: 589–594, illus.

(20) RANGASWAMI AYYANGAR, G. N., VIJIARAGHAVAN, C., SANKARA AYYAR, M. A., and PANDURANGA RAO, V.
1933. INHERITANCE OF CHARACTERS IN SORGHUM—THE GREAT MILLET. III. GRAIN COLOURS: RED, YELLOW, AND WHITE. Indian Jour. Agr. Sci. 3: 594–604, illus.
(21) ——— VIJIARAGHAVAN, C., SANKARA AYYAR, M. A., and PANDURANGA RAO, V.
1933. STUDIES IN SORGHUM—THE GREAT MILLET. II. ANTHER, STIGMA AND GRAIN COLOUR AFFINITIES. Indian Jour. Agr. Sci. 3: 605–608, illus.
(22) ——— VIJIARAGHAVAN, C., SANKARA AYYAR, M. A., PANDURANGA RAO, V., and SUBRAMANYAM, P.
1934. INHERITANCE OF CHARACTERS IN SORGHUM—THE GREAT MILLET. IV. BROWN GRAINS. Indian Jour. Agr. Sci. 4: 81–89.
(23) REED, GEORGE M.
1930. A NEW METHOD OF PRODUCTION AND DETECTING SORGHUM HYBRIDS. Jour. Heredity 21: 133–144, illus.
(24) SIEGLINGER, JOHN B.
1924. SEED-COLOR INHERITANCE IN CERTAIN GRAIN-SORGHUM CROSSES. Jour. Agr. Research 27: 53–64.
(25) ———
1933. INHERITANCE OF SEED COLOR IN CROSSES OF BROWN-SEEDED AND WHITE-SEEDED SORGHUMS. Jour. Agr. Research 47: 663–667.
(26) ——— SWANSON, A. F., and MARTIN, J. H.
1934. INHERITANCE OF AWN DEVELOPMENT IN SORGHUMS. Jour. Agr. Research 49: 663–668, illus.
(27) SWANSON, ARTHUR F.
1928. SEED-COAT STRUCTURE AND INHERITANCE OF SEED COLOR IN SORGHUMS. Jour. Agr. Research 37: 577–588, illus.
(28) ——— and PARKER, JOHN H.
1931. INHERITANCE OF SMUT RESISTANCE AND JUICINESS OF STALK IN THE SORGHUM CROSS, RED AMBER × FETERITA. Jour. Heredity 22: 51–56, illus.
(29) VINALL, H. N., and CRON, A. B.
1921. IMPROVEMENT OF SORGHUMS BY HYBRIDIZATION. Jour. Heredity 12: 435–443, illus.
(30) WOODWORTH, C. M.
1936. INHERITANCE OF SEEDLING STEM COLOR IN A BROOMCORN-SORGHUM CROSS. Jour. Amer. Soc. Agron. 28: 325–327.

FURTHER INVESTIGATIONS OF BROWN-STAINING FUNGI ASSOCIATED WITH ENGRAVER BEETLES (SCOLYTUS) IN WHITE FIR [1]

By ERNEST WRIGHT [2]

Associate pathologist, Division of Forest Pathology, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

In a previous paper (*13*)[3] the writer reported an apparently constant occurrence of a pronounced brown stain associated with egg galleries of *Scolytus ventralis* Lec. in white fir (*Abies concolor* Lindl. and Gord.). This stain was found to be caused by a fungus identified as *Trichosporium symbioticum* Wright.

A somewhat similar stain was later observed associated with the egg galleries of two other species of engraver beetles, *Scolytus praeceps* Lec. and *S. subscaber* Lec. This stain was a lighter brown and was usually less evident than that caused by *Trichosporium symbioticum*. Both stains are shallow and are confined to the current annual ring. The studies reported here were made to determine the cause of the lighter coloration, i. e., whether it is due to somewhat different environmental conditions occasioned by location or to another fungus.

These investigations were confined to white fir in California, although red fir (*Abies magnifica* Murr.) and other western evergreens are also known to be infested with these engraver beetles (*11*).

HABITS OF THE BEETLES

A brief description of the habits of the different beetles appears desirable for a better understanding of the fungus associations. More complete information can be found in a recent publication by Struble (*11*).

The galleries of the three different species of *Scolytus* vary in size, extent, and design, and can therefore be readily distinguished. The egg galleries characteristically groove the inner bark and adjacent sapwood. *Scolytus ventralis* egg galleries are confined to the cambial region of white fir trunks and generally occur where the diameter of the tree is more than 4 inches. It is not uncommon, however, for the tops of these same trees to be attacked by *Scolytus praeceps*. The egg galleries of the latter species are likewise found in the cambial

[1] Received for publication June 6, 1938; issued December 1938.

[2] Parts of the studies reported herein were carried on in direct cooperation wirh the Division of Forest Insect Investigations, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, and it is a pleasure to acknowledge the assistance of members of that Division, particularly George R. Struble. Grateful acknowledgment is also made to Dr. Lee Bonar, of the botany department, University of California, for his aid in determining the identity of the staining fungus.

[3] Italic numbers in parentheses refer to Literature Cited, p. 772.

region, but usually where the diameter of the tree is less than 4 inches (fig. 1). Large limbs and sapling white firs are also infested by this beetle. The third species, *S. subscaber*, prefers gnarled and mistletoe-infected branches in which to establish broods. The larvae of all three species characteristically feed in the stained areas surrounding the egg galleries. *S. ventralis* is considered of major importance and frequently causes the death of true firs. Infestations of *S. praeceps* and *S. subscaber*, particularly the latter, appear somewhat less important.

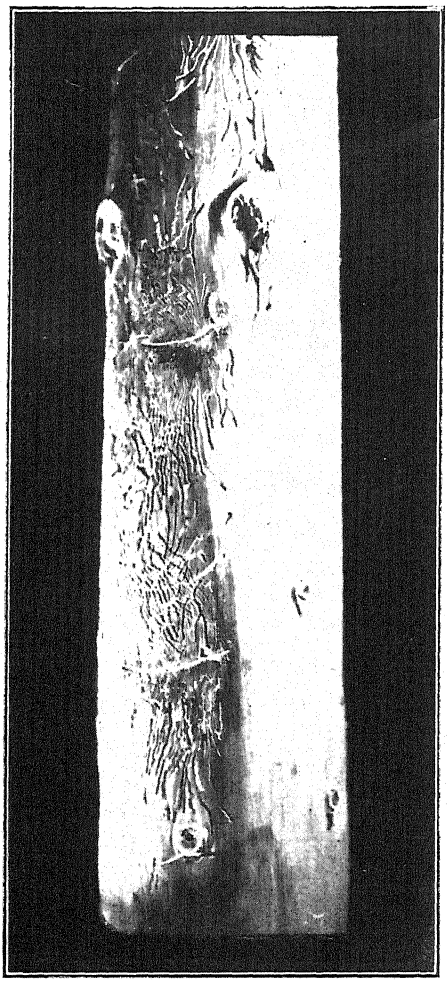


FIGURE 1.—Top section of an infested white fir tree with bark removed to show the stain associated with *Scolytus praeceps* galleries. (Approximately one-third actual size.)

ISOLATION RESULTS

Isolations were secured from stained wood and bark surrounding attacks of *Scolytus praeceps* and *S. subscaber by* cutting away surface contamination with a flamed scalpel. Individual pieces were then removed aseptically and placed on malt agar contained in Petri dishes. Transfers were made later into test-tube slants. Table 1 presents the results of these isolations.

The data presented indicate a fairly consistent fungus association. *Trichosporium symbioticum* was obtained in these isolations only when *Scolytus ventralis* galleries were present in areas adjacent to *S. praeceps* broods at points where the diameter of the tree approached 4 inches. Here the galleries of the two species of beetles sometimes intermingle. These brown stains apparently occur commonly only in association with the respective *Scolytus* beetle galleries.

Fructification of the *Spicaria* staining fungus occurs regularly within the beetle galleries in the form of small, whitish masses of spores. These spore aggregations can be readily mistaken for fresh *Scolytus* beetle frass. The fungus isolated appears entirely different in culture from *Trichosporium symbioticum*, macroscopically as well as microscopically.

TABLE 1.—*Summary of isolations from Scolytus praeceps and S. subscaber attacks in white fir over a 3-year period*

Species of beetle and part of tree attacked	Location of trees	Trees	Beetle attacks from which cultures were made	Isolations showing—		
				Specific fungus	Other fungi	No organism
Scolytus praeceps:		*Number*	*Number*	*Number*	*Number*	*Number*
Inner bark and xylem.	Stanislaus National Forest	4	56	50	4	2
Do	Yosemite National Park	1	12	10	1	1
Inner bark	Sierra National Forest	1	8	8	0	0
Total		6	76	68	5	3
Scolytus subscaber:						
Xylem	Tahoe National Forest	1	12	9	1	2
Inner bark	Sierra National Forest	1	6	4	2	0
Total		2	18	13	3	2

IDENTITY OF STAINING FUNGUS AS SPICARIA ANOMALA

TAXONOMY

The staining fungus isolated has been classified as belonging to the Verticilliae. The writer considers it to be *Spicaria anomala* (Corda) Harz. In 1838 Corda (*2, v. 2, p. 18, table 11, fig. 75*) named the fungus *Penicillium anomalum* and illustrated it. He originally found the organism on splinters of coniferous wood near Prague. Harting (*4*) later created the genus *Spicaria* (1846). In 1871 Harz (*5*) transferred several of Corda's species of *Penicillium* to the new genus and was the first to comment on the characteristic divergent spore chains. The fungus isolated in the present study does not appear to be the more commonly reported species, *Spicaria elegans* (Corda) Harz, since the upright conidiophores are shorter and contain fewer septa. So far as the writer is aware, this appears to be the first published report on the occurrence of *S. anomala* in association with *Scolytus praeceps* and *S. subscaber* egg galleries.

In designating this fungus *Spicaria anomala*, the writer desires to emphasize the fact that considerable variation was found in the number of main branches that extend from the conidiophore stalks. In this respect the isolates from white fir appear atypical when compared with Corda's illustrations. The variations are not considered sufficient, however, to warrant the establishment of a new species or even a strain.

MORPHOLOGICAL CHARACTERISTICS

Spicaria anomala has already been adequately described, and only the important characteristics of the species will be given here as applying to isolates from white fir. Under the low power of a binocular microscope (20 ×), the upright stalks with the accompanying chains of spores resemble miniature, white, bushy trees. Under higher power (40 ×) the branches are seen to occur verticillately in threes around the conidiophore stalks with ultimate ramuli also generally in threes. The spores are borne in great profusion as single chains

from the apex of each ramulus. Individual spores are hyaline, ovate, and 5μ to 6μ in diameter. In potato-dextrose agar (pH 5.5) the submerged hyphae of *S. anomala* are plainly septate, and in old cultures (5 months) attain a maximum width of 5.5μ, averaging approximately 3.0μ. Aerial conidiophore stalks average about 3.5μ in diameter, with branches occurring at a height of 20μ to 40μ. The stalks are usually not more than five-septate. The total height of the conidiophores with chains attached is approximately 150μ (fig. 2).

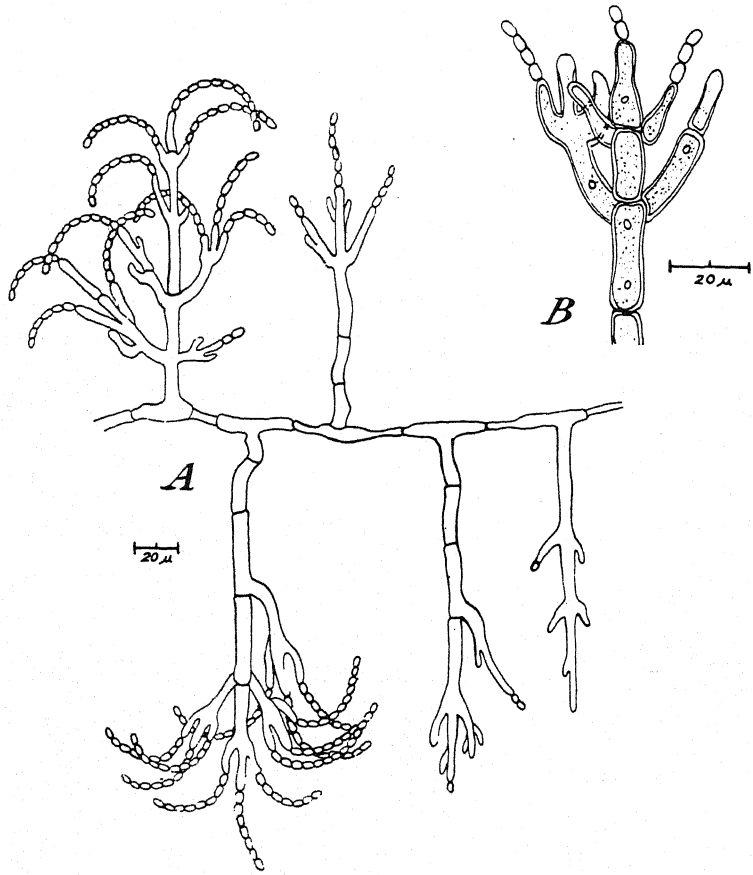


FIGURE 2.—Diagrammatic drawings of *Spicaria anomala* (Corda) Harz grown on potato-dextrose agar (pH 5.5). *A*, Mature and immature aerial conidiophores showing typical branching, septations, and ramuli formations with catenulate spores. Normally the branches are whorled verticillately around the conidiophore stalks. *B*, Details of conidiophore, illustrating the manner of spore attachment to the apex of each ramulus.

Spicaria anomala formed white to cream-colored, zonate colonies and readily produced conidiophores and chains of conidia on all the media on which it was grown (malt, potato-dextrose, corn-meal, and Czapek's agars) (fig. 3). A brown coloration was imparted to the

medium, particularly to potato-dextrose agar, increasing in intensity with the age of the culture. Since the hyphae remained hyaline, the coloration of the agar probably was due to the break-down of certain nutrients. Coloration was less intense in the other agars used. It may be significant that *Trichosporium symbioticum* hyphae were colored brown most readily in malt agar and less readily in potato-dextrose agar.

Microscopic examinations of sections of infected wood stained by the Cartwright method (*1*) showed the hyphae of *Spicaria anomala* in the tracheids and medullary ray cells. No conidiophore formation was seen, however, within the tracheids as reported for *Trichosporium symbioticum*. The hyphae were very delicate as compared with those of other staining fungi, but were well distributed throughout the

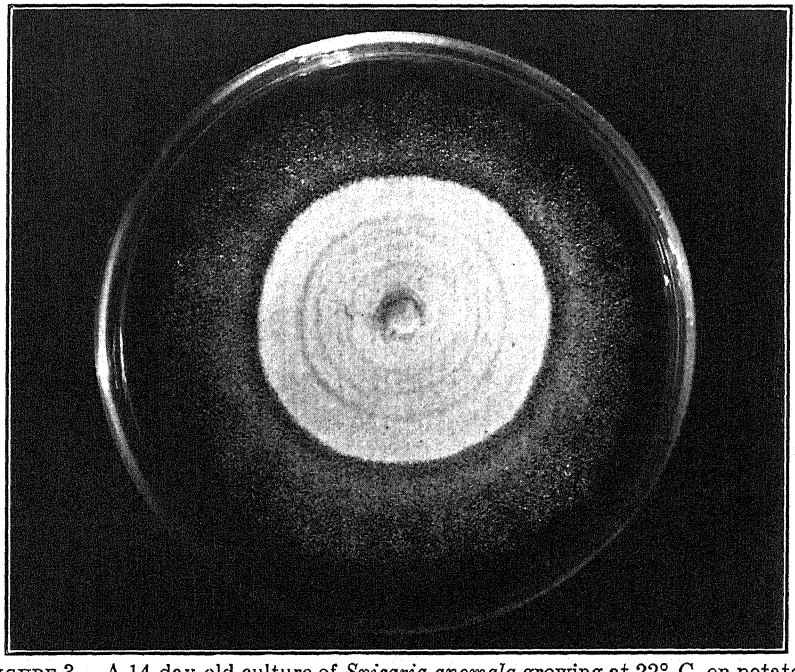


FIGURE 3.—A 14-day-old culture of *Spicaria anomala* growing at 22° C. on potato-dextrose agar, illustrating typical zonations. Three-fourths actual size.

stained areas. They regularly entered the medullary rays and tracheids through the pits. They did not appear, however, to concentrate in the ray parenchyma to the same extent as do the blue-staining fungi (*Ceratostomella* spp.). No method of differential staining that clearly showed the presence of the fungus hyphae in the phloem was found; therefore isolations proved the only reliable evidence of their presence in these tissues.

SCOLYTUS BEETLES AS CARRIERS OF STAINING FUNGI

To determine to what extent *Scolytus praeceps* adults carry *Spicaria anomala*, a number of these beetles were collected as they emerged from infested logs contained in entomological cages. They were dropped individually into separate Petri dishes containing lukewarm

agar, where they swam about vigorously until the agar solidified. Later the beetles were removed with sterile forceps, and the plates were incubated. The fungus colonies secured were identified microscopically, and it was found that 66 out of 75 plates contained the specific fungus *S. anomala*. In six of the plates an overgrowth of molds retarded the growth of other fungi, while three of the plates remained sterile. Figure 4 shows colonies of *S. anomala* secured

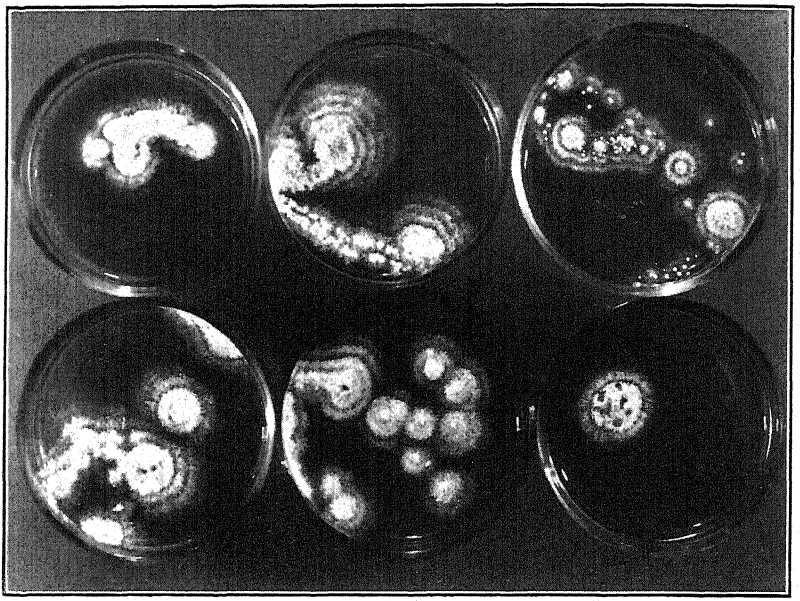


FIGURE 4.—Six Petri dish cultures secured from individual *Scolytus praeceps* beetles at time of emergence. The beetles were dropped into lukewarm agar, where they swam about vigorously until it solidified.

directly from *Scolytus praeceps* beetles in the manner described above. Similar tests were not made with *S. subscaber*, since no adults could be obtained.

The above-mentioned cultural isolations indicate that adult *Scolytus praeceps* beetles carry and regularly introduce *Spicaria anomala* into their egg galleries. Thus, they actually inoculate the cambial region of infested trees, and by the time the larvae begin to feed the stain is well developed. No attempt was made to determine whether the beetles carried the fungus internally or externally. These results, together with the isolations secured from the stained inner bark and xylem, suggest that *S. anomala* is probably the predominant fungus associated with the beetles. Since *Trichosporium* has been previously shown to occur in association with *S. ventralis*, these results, together with certain others obtained outside the present studies, suggest that the relationships are specific and not a matter of chance.[4] Similar conclusions have also been advanced by investigators reporting on other fungi associated with different species of beetles (*7*, *9*, *10*).

[4] In a preliminary investigation of brown-staining fungi associated with the egg galleries of *Scolytus* beetles infesting Douglas fir (*Pseudotsuga taxifolia* (Lam.) Britton), the writer secured a number of isolations of *Trichosporium* sp. from the stained wood surrounding *Scolytus unispinosus* Lec. broods. *Spicaria* sp. was

PATHOGENICITY TESTS

A number of inoculation tests were made to determine the pathogenicity of *Spicaria anomala* and to compare the rate of stain spread with that caused by *Trichosporium symbioticum*.

By drilling horizontally between the bark and the wood with a $\frac{3}{32}$-inch bit, it was possible to approximate the size and position of the beetle galleries in white fir trunks. The inoculum, consisting of bits of mycelium in agar obtained from pure cultures of *Spicaria anomala*, was inserted into the drill openings. Thus the fungus was placed in direct contact with the cambial region of the trees at diameters ranging from 4 to 9 inches. One hundred inoculations were made in the opposite side of the same standing white firs that had been previously inoculated with *Trichosporium symbioticum*.

All of the inoculations resulted in infection, and at the end of 7 or 8 weeks the average visual longitudinal spread of the *Spicaria* stain in 10 different trees was found to be 3.5 mm per week. This was approximately one-half as fast as the spread of *Trichosporium symbioticum* stain in the same trees. Koch's postulates were followed in a number of cases to prove more definitely that *S. anomala* causes a stain similar to that of *T. symbioticum*. A total of 100 checks made with sterile agar showed no stain development. It was also observed that *S. anomala* killed the cambium as the mycelium advanced, as was concluded for *T. symbioticum*. Both fungi therefore appear to aid the beetles in killing infested trees or parts of trees.

PHYSIOLOGICAL TESTS

Since these studies indicate that the respective staining fungi are associated with certain species of *Scolytus*, it is possible that physiological conditions in the different portions of the tree infested may in some manner favor the establishment of one fungus to the disadvantage of other organisms. Preliminary laboratory and field tests were made in an attempt to analyze some of the possible factors of the environment. The tests were conducted on a comparative basis to determine the differences between the development of *Spicaria anomala* and *Trichosporium symbioticum* under conditions as similar as possible.

EFFECT OF AERATION

An anaerobic apparatus was employed in which pyrogallic acid and potassium hydroxide were used to exhaust the oxygen from a closed container in the usual manner (*3*). Three Petri dishes containing malt agar inoculated with *Spicaria anomala* and three with *Trichosporium symbioticum* were placed on a shelf in this chamber. The covers were not removed from the dishes. After a lapse of 1 week at room temperature (22°–25° C.), radial growth measurements were made.

Under the conditions of these reduced aeration tests, the two species of fungi showed reduction in growth. The growth of *S. anomala* was less adversely affected than that of *T. symbioticum*, but the difference did not appear significant.

EFFECT OF TEMPERATURE

Differences in temperature tolerance might also explain to some extent the restricted occurrence of the stains in the respective parts

of the tree. In the present investigation it was not feasible to make seasonal measurements on the influence of bark thickness and color on the temperature in the cambial region of white fir. However, the effect of temperature on the growth of the respective fungi was compared in the laboratory on the same culture medium.

A series of tests was run in which both organisms were grown on malt agar and subjected to constant temperatures of 5°, 15°, 25°, and 30° C. The results indicate that *Spicaria anomala* is more restricted in temperature range than *Trichosporium symbioticum*. In no case did the growth of *S. anomala* equal that of *T. symbioticum* (fig. 5). Fluctuating temperatures of −15° to +26° C. also favored the growth of *Trichosporium* over that of *Spicaria*.

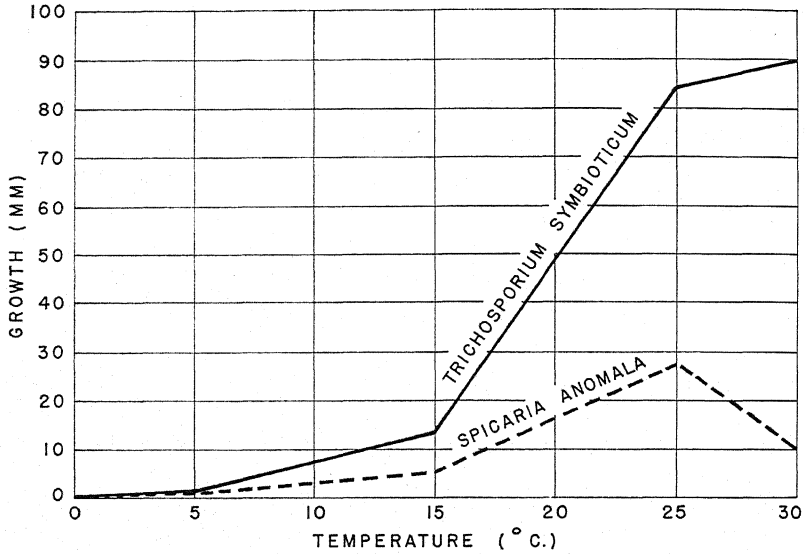


FIGURE 5.—Radial growth of *Trichosporium symbioticum* and *Spicaria anomala* at different temperatures on malt agar for 1 week. (On the basis of measurements secured from triplicate plates subjected to the temperatures of 5°, 15°, 25°, and 30° C.)

The lack of supporting field data makes it difficult to correlate these results with natural conditions. On the basis of these tests, however, the effect of temperature on the growth and development of the respective fungi does not appear to be significant.

EFFECT OF ACIDITY

The development of the respective fungi in solid media adjusted to different degrees of acidity showed that *Trichosporium symbioticum* makes optimum growth at pH 5.0 to 6.0, while *Spicaria anomala* grows best at pH 7.0. In all the acidity tests made, however, the radial growth of *T. symbioticum* always exceeded that of *S. anomala*.

EFFECT OF MOISTURE

Since moisture has been shown to influence the growth of blue-staining fungi by altering the oxygen content of the wood (*8*), it seemed advisable to investigate the comparative growth rates of

Spicaria anomala and *Trichosporium symbioticum* under various moisture conditions. This was done in the field in the following manner:

Five living white firs were partly girdled by chopping a 2-inch-wide band into the wood on one side of the tree, thus stopping normal conduction of moisture on that side. In the same way, five other trees were completely girdled by chopping a similar band entirely around the trunk. Twenty drill inoculations were made in each tree, above the partial or complete girdles, *S. anomala* and *T. symbioticum* being used for 10 inoculations each. The respective inoculations were arranged in two vertical rows on the same side of the trunk. The inoculum, in each case consisting of a small piece of malt agar containing mycelium of the respective fungus, was inserted into the drill holes with sterile needles.

After 2 months the development of the two stains was determined by removing the bark and measuring the average longitudinal spread of each stain above and below the points of inoculation.

To determine the amount of moisture present in the inoculated part of each tree, three samples were extracted from the unstained wood between the two vertical rows of inoculations by means of a 1-inch arch punch.[5] One sample was obtained from the top, another midway, and the third at the bottom of the inoculated regions. In extracting the samples the arch punch was driven through the bark into the wood to a depth of several annual rings. The samples were removed within the circular blade of the arch punch when it was withdrawn. Each sample was immediately wrapped in tin foil and placed in a screw-top container to prevent desiccation before the initial weighing could be made. The bark was removed, and each sample was trimmed at the time of weighing to the thickness of the current annual ring, since, as previously stated, this is the depth of penetration of both stains. The wood cores were then dried at 100° C. until a constant weight was obtained, and the percentage of moisture was calculated on the basis of dry weight. Moisture determinations were made at the time of inoculation and at the end of the tests.

These tests indicated that the maximum visual spread of *Trichosporium symbioticum* stain took place when the current annual ring contained approximately 165 percent moisture, while 155 percent appeared to be the optimum amount of moisture for *Spicaria anomala*. When the moisture was greater than 155 percent, *S. anomala* stain advanced less rapidly in longitudinal spread than did that of *T. symbioticum*.

At the higher moisture contents, however, the brown coloration of *Spicaria anomala* stain was practically equal to that of *Trichosporium symbioticum*. This indicates that the lighter color in association with the *Scolytus praeceps* and *S. subscaber* galleries may be correlated directly with reduced moisture content of the infested parts. When the moisture was reduced to 120 percent, very little evidence of stain could be detected for either species.

The moisture percentages given above are about the same as those reported to favor the development of blue-staining fungi in pine and spruce (*6*). Ordinary blue-staining fungi (*Ceratostomella* spp.) are uncommon, however, in true firs.

[5] An arch punch is a tool used in the leather trade, consisting of a hollow, circular, steel blade with a cutting edge over which a steel handle arches.

EFFECT OF NUTRITION

To study the influence of nutrition on growth, the two fungi were introduced into Petri dishes containing a starvation agar to which various carbohydrates or nitrogen sources had been added. The basic agar was made up as follows:

Magnesium sulphate ($MgSO_4.7H_2O$)	gram	1. 0
Monopotassium phosphate (KH_2PO_4)	do	1. 0
Calcium sulphate ($CaSO_4.2H_2O$)	do	. 5
Agar	do	20. 0
Distilled water	liter	1

Eight carbohydrate and seven nitrogen sources were included in these tests. The results obtained from average measurements of the diameter of the colonies in triplicate trials for each compound tested are presented graphically in figure 6.

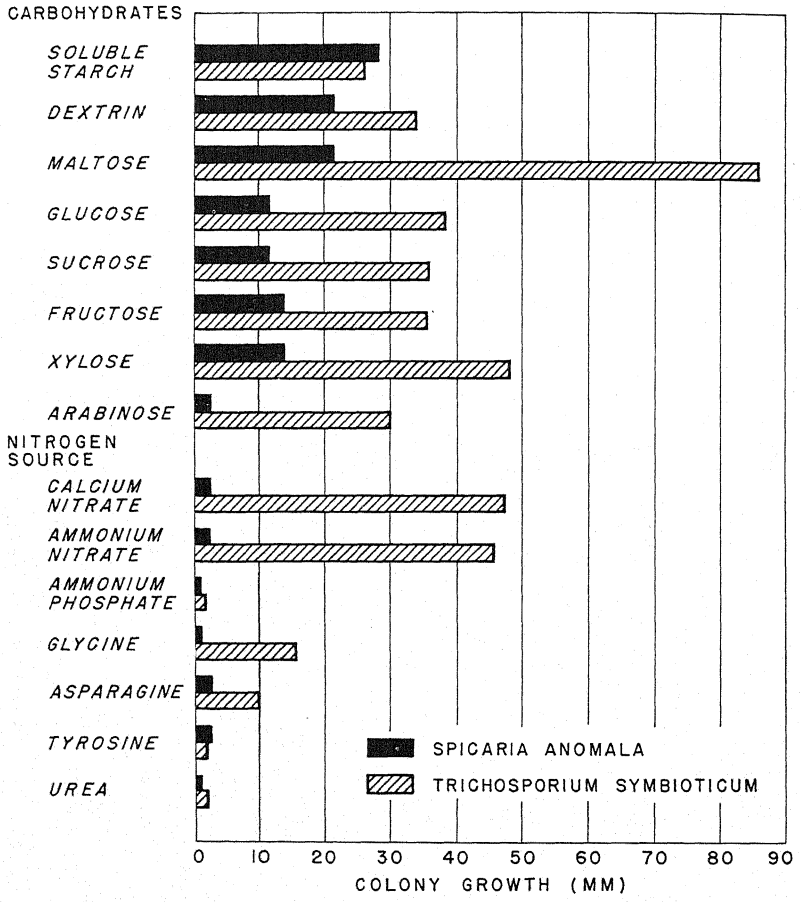


FIGURE 6.—Diagrammatic comparisons of the colony growth of *Spicaria anomala* and *Trichosporium symbioticum* on various nutrient agars at 25° C. on the basis of measurements from triplicate replications over a 10-day period. All media adjusted to pH 6.0.

It may be significant that *Spicaria anomala* made the best growth on the medium containing starch, and the next best growth on the media containing the intermediate hydrolysis sugars, dextrin and maltose. Relatively poor growth was made on the glucose medium. On the other hand, *Trichosporium symbioticum* grew very poorly on starch, made only fair growth on dextrin, and made the best growth on the maltose medium. For both organisms, sporulation was most pronounced in the medium containing the pentose, xylose.

These results indicate that the hydrolysis of polysaccharides is more necessary for good growth of *Trichosporium symbioticum* than of *Spicaria anomala*. Just how significant this might be is not known, since the condition of the trees, seasonal variations, time of infestation, and many other factors may influence nutritional balances. These differences in the utilization of carbohydrates may partially explain how the two fungi can grow and intermingle in the same areas without direct nutritional competition at diameters of about 4 inches where sometimes both *Scolytus ventralis* and *S. praeceps* galleries occur. This might be appropriately termed the region of transition on the trunk, above and below which the respective stains are commonly found alone.

On all the media where there was appreciable growth, with the exception of the starch medium, the development of *Trichosporium symbioticum* has always exceeded that of *Spicaria anomala*.

Spicaria anomala did not appear to utilize nitrogen as readily as did the *Trichosporium*. However, even for the latter fungus the inorganic forms of nitrogen were better. Little growth occurred in the media containing organic nitrogen, but of these the simplest amino acid, glycine, was most favorable. The carbohydrate-nitrogen ratio may also be of importance, although other cultural trials not presented here did not show significant differences in this regard.

On the basis of these tests it appears that physiological conditions probably do not especially favor the establishment of one staining fungus over the other. Instead, it seems most likely that *Spicaria anomala* and *Trichosporium symbioticum* commonly occur in definite regions of the tree only because each fungus is transported there separately by the respective beetles.

INFLUENCE OF THE FUNGI ON THE ENVIRONMENT

It seems possible that *Spicaria anomala* and *Trichosporium symbioticum* may have different influences on certain environmental factors that may favor particularly the respective beetle associations. Several investigators have already indicated independently that wood-staining fungi associated with various beetles may be definitely beneficial, since they create drier environmental conditions in which the larvae develop (*7*, *9*, *13*). To determine the effect of *S. anomala* and *T. symbioticum* on the moisture content of the respective stained regions, a series of tests was made as follows.

Ten white firs 3 to 12 inches in diameter at breast height were selected, and four sets of inoculations were made in each tree. In each set a ¼-inch cork borer (*12*) was employed to remove five bark disks arranged in double bands, one below the other, 2 inches apart.

Three borings, one-half inch apart, were made in the top band with two in the lower band in alternation with those above. Mechanical girdling was thus avoided. Two sets of borings made on opposite sides of the tree were inoculated with *Spicaria anomala* and two with *Trichosporium symbioticum*. The inoculations were made by removing each disk with the cork borer and inserting a bit of agar that contained the mycelium of the respective fungus, after which the disk was immediately replaced. When a band of inoculations was completed, the disks were held in place and desiccation was prevented by covering them with adhesive tape. The inoculum in all cases came from pure cultures.

Two weeks after these inoculations were made samples of the respective stains, one from each set, were extracted between the inoculation bands by means of the arch punch previously described. Duplicate samples of unstained wood were taken from between the inoculation sets. Moisture contents were determined on the basis of the average dry weights of stained and unstained duplicate samples. The green weights of the samples were obtained immediately after extraction to eliminate variations due to air drying. The samples were finally dried at 100° C. until a constant weight was reached. The average weights and the percentages of moisture in the samples are given in table 2.

TABLE 2.—*Moisture content of stained and unstained white fir wood 2 weeks after inoculation (current annual ring only)*

Diameter breast high (inches)	Unstained wood			*Trichosporium*-stained wood			*Spicaria*-stained wood		
	Wet weight	Dry weight	Moisture [1]	Wet weight	Dry weight	Moisture [1]	Wet weight	Dry weight	Moisture [1]
	Grams	*Grams*	*Percent*	*Grams*	*Grams*	*Percent*	*Grams*	*Grams*	*Percent*
3	0.757	0.490	54	0.945	0.607	56	0.922	0.601	53
4	.902	.571	58	.654	.456	43	.735	.522	41
4	.982	.512	92	.601	.398	51	.730	.499	46
5	1.072	.445	141	1.076	.513	109	.833	.496	68
5	1.305	.519	151	.871	.428	104	.873	.468	87
6	1.325	.476	178	1.129	.564	100	1.127	.678	66
9	1.341	.518	159	.857	.468	83	.856	.590	45
10	1.157	.459	152	1.048	.529	98	.819	.524	56
11	1.642	.527	211	.910	.442	106	.926	.515	80
12	1.738	.565	208	1.324	.613	116	1.158	.621	86
Average	1.222	.508	140	.941	.502	86	.898	.551	63

[1] Percentage of moisture based on dry weight.

It should be noted that wood stained by *Trichosporium symbioticum* contained only three-fifths, and the *Spicaria*-stained wood less than half of the moisture found in unstained wood of the same trees. Unstained wood of white firs 4 inches and less in diameter, the size at which *Scolytus praeceps* infestations most commonly occur, had the relatively low average moisture content of 68 percent. Even in these trees the *Spicaria* stain reduced the amount of moisture nearly one-third. The wood of trees greater than 4 inches in diameter, similar in size to those attacked by *Scolytus ventralis*, lost two-fifths of its moisture when stained by the *Trichosporium* fungus associated

with this beetle. In all trees, however, the *Trichosporium* stain dried the wood less than the *Spicaria* stain.

These differences in the amount of moisture present in the wood stained by the two fungi suggested that the respective beetle broods may have different moisture requirements. The larvae of *Scolytus praeceps* appear to require less moisture for development than do those of *S. ventralis*. Each fungus may therefore serve to maintain a favorable moisture balance in infested areas for the broods of associated beetles.

DISCUSSION

The inoculations reported above demonstrate that both *Trichosporium symbioticum* and *Spicaria anomala* can develop in white firs up to 13 inches in diameter. Since neither fungus is apparently restricted by the size of the tree and physiological tests did not reveal any outstanding inhibitions, the inference may be drawn that the respective fungi are confined naturally to separate parts of the tree only because they are introduced there by specific beetles. Isolation results showing that the different species of *Scolytus* adults regularly carry only one staining fungus support this inference.

The association of the stains with respective beetle broods therefore may be of benefit in several ways. *Trichosporium symbioticum* kills the cambium in advance of the development of *Scolytus ventralis* larvae, as was first observed by Struble (*11*). Field observations and cultural results reported here indicate that *Spicaria anomala* in a similar way precedes the galleries of *Scolytus praeceps* and *S. subscaber*. Thus a barrier is provided against the inflow of resin, which might otherwise drown out the broods. This may be of vital importance to the beetles in another respect, since Struble has also observed that white fir resin acts both as a repellent and a toxin to *S. ventralis* adults. Whether the resin has a similar effect on the larvae is not known, but the restriction of resin flow at the time the beetles emerge may be of the utmost importance. The drying of the wood can also influence the environment for the respective beetle brood by correspondingly altering the oxygen content and reducing heat and cold conduction.

It appears from these tests that the presence of the commonly associated fungus that causes the staining of the wood adjoining the egg galleries may well be indispensable to the development of the brood. This has not yet been positively demonstrated by direct experimentation. Likewise, the optimal and limiting factors for brood development and food supply are as yet undetermined. The tests completed, however, suggest a beneficial relationship between the beetles and definite staining fungi.

SUMMARY

Three species of scolytid beetles commonly infest white fir (*Abies concolor*), namely, *Scolytus ventralis*, *S. praeceps*, and *S. subscaber*. The galleries of these beetles are confined to the cambial region, but are characteristically formed in the base, the top, and the branches of the tree respectively.

Brown discolorations have been frequently observed to be regularly associated with the galleries, appearing somewhat less pronounced with those of *S. praeceps* and *S. subscaber*.

Isolations from the stained bark and adjacent sapwood indicate that two different fungi cause the stains. Isolations from newly emerged beetles further suggest definite specificity. The fungus most commonly associated with *Scolytus praeceps* and *S. subscaber* has been identified as *Spicaria anomala*, while *Trichosporium symbioticum* causes a darker discoloration and has been shown previously to be constantly associated with *Scolytus ventralis*.

Inoculations have demonstrated that both *Spicaria anomala* and *T. symbioticum* are pathogenic stains that kill the cambium as they advance. In this way they may aid the beetles in overcoming infested trees.

The respective fungi appear to have a definite influence on the immediate environment. It was found that *S. anomala* reduced the moisture content of the stained wood to a greater extent than did *T. symbioticum*, which in turn reduced the moisture on an average to three-fifths of that of unstained wood.

Physiological studies showed some differences between the two staining fungi, but these do not seem significant. It appears most likely that the respective fungi occur in definite regions of the tree because they are introduced into the egg galleries by the different *Scolytus* beetles.

On the basis of the evidence secured, the association of the brown-staining fungi with the respective beetles may well be of definite aid in the successful establishment and maintenance of individual beetle broods.

LITERATURE CITED

(1) Cartwright, K. St. G.
1929. A satisfactory method of staining fungal mycelium in wood sections. Ann. Bot. [London] 43: 412–413.

(2) Corda, A. C. I.
1838. Icones fungorum hucusque cognitorum. v. 2, 43 pp., illus. Pragæ.

(3) Haldane, J. S.
1920. Methods of air analysis. Ed. 3, 147 pp., illus. London.

(4) Harting, Pieter.
1846. Recherches sur la nature et les causes de la maladie des pommes de terre en 1845. Nieuwe Verhandl K. Nederland. Inst. Wetensch. 12: 203–297, illus. [Original not seen.]

(5) Harz, Carl Otto.
1871. Einige neue hyphomyceten Berlin's und Wien's, nebst beiträgen zur systematik derselben. Bull. Soc. Imp. Nat. Moscow 44(1): 88–147, illus. [Original not seen.]

(6) Lagerberg, T., Lundberg, G., and Melin, E.
1927. Biological and practical researches into blueing in pine and spruce. Svenska Skogsvårdsför. Tidskr. 25: 561–739, illus. [Résumé in Swedish, pp. 692–739.]

(7) Leach, J. G., Orr, L. W., and Christensen, Clyde.
1934. The interrelationships of bark beetle and blue-staining fungi in felled Norway pine timber. Jour. Agr. Research 49: 315-342, illus.

(8) Münch, E.
1908. Die blaufäule des nadelholzes. Naturw. Ztschr. Forst u. Landw. 6: [297]–323, illus.

(9) Nelson, Ralph M.
1934. Effect of bluestain fungi on southern pine attacked by bark beetles. Phytopath. Ztschr. 7: [327]–353, illus.

(10) RUMBOLD, CAROLINE T.
1931. TWO BLUE-STAINING FUNGI ASSOCIATED WITH BARK-BEETLE INFESTATIONS OF PINES. Jour. Agr. Research 43: 847–873, illus.
(11) STRUBLE, G. R.
1937. THE FIR ENGRAVER BEETLE, A SERIOUS ENEMY OF WHITE FIR AND RED FIR. U. S. Dept. Agr. Cir. 419, 16 pp., illus.
(12) WRIGHT, ERNEST.
1933. A CORK-BORER METHOD FOR INOCULATING TREES. (Phytopath. Note.) Phytopathology 23: 487–488.
(13) ———
1935. TRICHOSPORIUM SYMBIOTICUM, N. SP., A WOOD-STAINING FUNGUS ASSOCIATED WITH SCOLYTUS VENTRALIS. Jour. Agr. Research 50: 525–538, illus.

COMPLETE OR PARTIAL INHIBITION OF FLOWERING IN CERTAIN PLANTS WHEN DAYS ARE TOO SHORT OR TOO LONG[1]

By H. A. ALLARD

Senior physiologist, Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

On the basis of their flowering behavior as related to or dependent upon length of day, it has long been recognized that plants may be grouped into three classes—the long-day plants, the short-day plants, and indeterminate plants, i. e., those insensitive to length of day. The long-day plants are induced to flower or flower more quickly as the days are lengthened and the short-day plants as the days are shortened; the indeterminate plants have been so called because their usual flowering appears to be little related to any particular length of day in the natural terrestrial arrangement.

Typical long-day, short-day, and indeterminate plants have been found in abundance. Further studies of the behavior of different plants in relation to length of day have shown that still other plants exist that flower within a definite range of length of day, producing flowers less readily or becoming strictly vegetative when the days are either sufficiently shortened on the one hand or sufficiently lengthened on the other. Since the flowering behavior of these plants in relation to length of day is so distinctive, they have been termed "intermediate" to distinguish them from the long-day, the short-day, and the indeterminate plants.

Relatively few plants showing intermediate flowering behavior have been found. Climbing hempweed (*Mikania scandens* (L.) Willd.) appears to be a typical example of this class, and several other wild plants, including the native wild bean (*Phaseolus polystachyus* (L.) BSP.) and the wild boneset (*Eupatorium torreyanum* Short.), likewise show more or less definite tendencies toward the same behavior. A variety of tropical sugarcane (28 N. G. 292) of the species *Saccharum spontaneum* L. gives evidence of having the sharpest and narrowest flowering range of any plant yet found in this group.

METHODS

In the tests where the entire plant was subjected to the treatment, constant daily light periods of 10, 12, 12½, 13, 13½, 14, 14½, 16, and 18 hours were used. The behavior of the plants in response to the full length of day is taken as the normal seasonal behavior. In those tests with light periods shorter than 16 hours, i. e., from 10 to 14½ hours, inclusive, the plants were kept in large well-ventilated lightproof houses to exclude daylight. The containers with the plants remained upon movable trucks running on tracks and were moved into the daylight on fixed schedules each morning and evening to secure the proper

[1] Received for publication June 20, 1938; issued December 1938.

daylight periods. In all tests involving light periods shorter than the maximum length of the summer day in the latitude of Washington, which is about 15 hours, the light source was natural daylight. However, in those tests requiring 16 and 18 hours of light, respectively, each day, it was necessary to make use of artificial light from sunset. This supplemental light in each test was obtained from four 200-watt incandescent bulbs with reflectors kept at a distance of 1 foot above the plants. In all tests the plants were grown in large galvanized-iron buckets of 14-quart capacity.

EXPERIMENTAL RESULTS

CLIMBING HEMPWEED

Climbing hempweed *(Mikania scandens)* is a perennial usually found in low-ground thickets and is distinctive in being the only species of climbing composite in the Washington region. The slender herbaceous stems die to the ground each autumn, leaving matted rootstocks from which new and vigorous shoots arise each year as soon as spring opens. The flowers also produce seed in abundance, and these tiny propagules, furnished with a hairy pappus, are readily carried away by the winds to establish new plants elsewhere in damp thickets.

Studies were made both with seedlings and with root divisions from a single large rootstock, and the behavior of the plants from these two sources will be presented.

BEHAVIOR OF SEEDLINGS

Seed of *Mikania scandens* was sown October 5, 1936, in a flat that was kept out of doors until December 7, 1936. The flat was then brought into a cool greenhouse ranging in temperature from 50° to 55° F. Germination took place January 1, 1937, and the small seedlings were pricked off into thumb pots March 3, 1937. Young, vigorous plants having an average height of about 3 inches were transferred to buckets, two in each container, May 18, 1937. On this date the tests in which only natural daylight was used began, while the tests of 16 and 18 hours, requiring supplemental artificial light, began April 22. The flowering behavior for the various constant daily light periods is shown in table 1.

TABLE 1.—*Flowering behavior of seedlings of Mikania scandens exposed to various constant daily light periods*

Daily light period (hours)	Date of budding	Date of blooming	Height of stem	Remarks
			Inches	
10	June 18	July 9	4	Very few flowers.
12	June 5	June 28	50	Few flowers and flowering soon ceased.
12½	June 10	...do...	50	Many flowers.
13	June 9	...do...	50	Do.
13½	...do...	...do...	50	Do.
14	June 11	June 26	50	Do.
14½	June 8	June 28	50	Do.
16	Aug. 19	Sept. 18	50	1 or 2 flower clusters only.
18 [1]				
Full day	June 22	Aug. 10	50	Many flowers.

[1] Plants failed to bud at this day length.

Behavior of Rootstock Divisions

Divisions from a single rootstock of *Mikania scandens* were potted in 3-inch pots in the cool greenhouse October 26, 1936. Shoots were starting January 21, 1937. These divisions were transferred to buckets, and the tests began May 18, 1937, when the plants were about 30 inches in height. The results are given in table 2.

Table 2.—*Flowering behavior of rootstock divisions of Mikania scandens exposed to various constant daily light periods*

Daily light period (hours)	Date of budding	Date of blooming	Height of stem	Remarks
			Inches	
10	June 30	July 24	50	Four flower clusters only.
12	June 7	July 6	50	Few flowers.
12½	June 11	July 2	50	Do.
13	June 8	June 28	50	Sparse flowering.
13½	June 7	June 25	50	Good flowering.
14	June 12	June 28	50	Do.
14½	June 7	---do----	50	Do.
16 [1]				
18 [1]				
Full day	June 21	July 5	50	Good flowering.

[1] Plants failed to bud at this day length.

The plants derived from rootstock divisions produced very few flowers on lengths of day of 10, 12, and 12½ hours. Flowering soon ceased, and the plants maintained a purely vegetative condition but were less inclined to become scrambling twiners than those that experienced longer light periods.

Localization Tests

Rootstock cuttings of one plant of *Mikania scandens* were made and placed in 3-inch pots October 26, 1936, in the cool greenhouse. These were starting growth January 21, 1937. In April the main stem was cut back to a node within a few inches of the ground in order to stimulate new branches from this node. The plants were transferred to buckets, and the tests began May 29 (fig. 1).

The cases used in these tests were so constructed that a branch conducted inside the case from the plant in the bucket placed just outside was completely excluded from light when the sides of the case were fastened in position. The inside branch was given 10 hours of light each day, i. e., from 6 a. m. to 4 p. m., while an opposite branch from the same node was exposed to the full length of day. Two tests were made. In one the bucket was placed inside the case, with one branch remaining inside and an opposite branch conducted outside; in the other the bucket was placed outside the case, with one branch remaining outside and an opposite branch conducted inside the case.

In the test with the bucket inside the case, the length of the inside branch was 15 inches and that of the outside branch 16 inches, when the test began. The inside branch, given 10 hours of daylight each day, produced buds June 17 and open flowers July 12 at a length of 40 inches. A single flower cluster appeared, and no further flowering took place, the branch otherwise remaining in a vegetative condition all summer. The stem exposed to the full length of day produced buds June 15 and open flowers July 8. This branch was very floriferous throughout the summer.

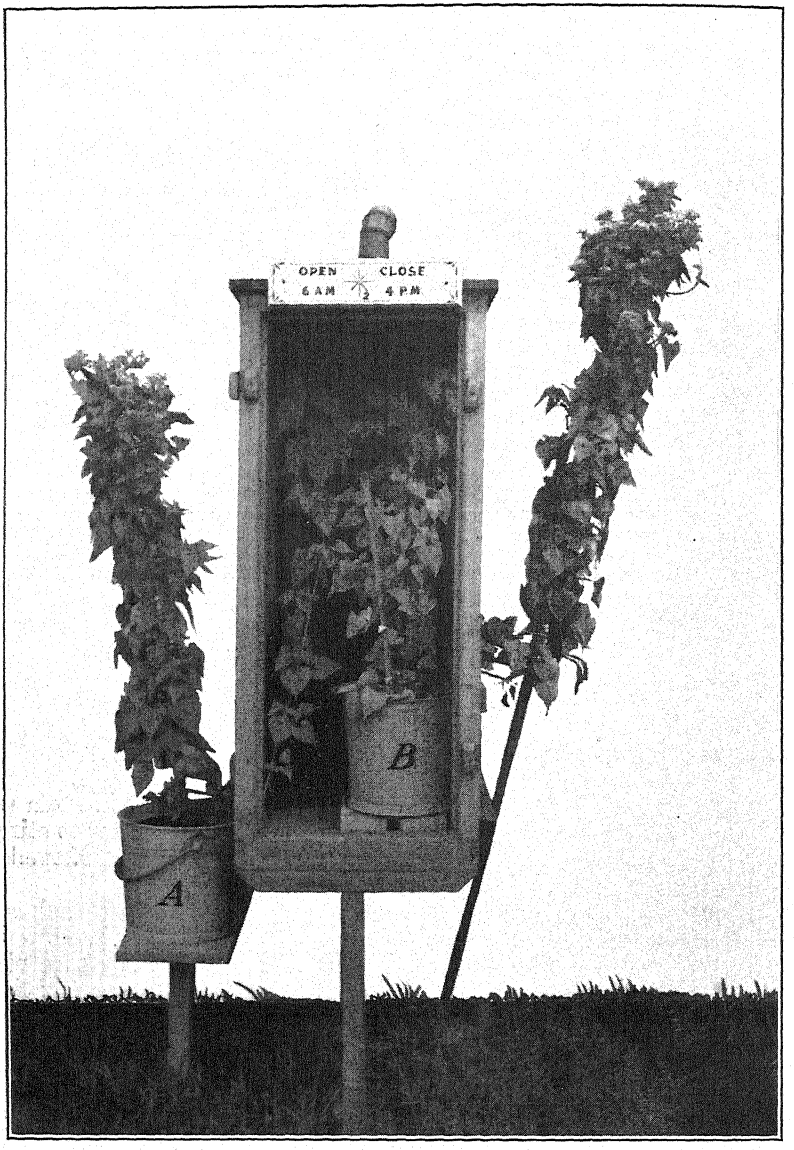


FIGURE 1.—Climbing hempweed (*Mikania scandens*) in localization tests begun May 29. Portions of the plants inside the case received 10 hours of daylight each day, those outside experienced the full day. *A*, plant with rooted portion outside under the full length of day, and one branch inside. The outside branch showed visible buds June 17 and flowered July 8, the flowers appearing in great abundance. The inside branch never budded visibly. *B*, Plant with rooted portion inside the case, and one branch outside under the full length of day. The inside branch produced one flower cluster only, July 12, then ceased. The outside branch produced buds June 15 and open flowers July 8; very floriferous throughout summer. These plants were grown from divided crowns. Photographed August 13.

In the test with the bucket outside the case, the length of the outside branch was 24 inches and that of the inside branch 13 inches, when the test began. The inside stem, experiencing a 10-hour day, never budded; the outside stem, receiving full daylight, showed buds June 17 and open flowers July 8, at 50 inches.

In both localization tests, the branch receiving only 10 hours of daylight either failed to flower entirely or produced a single cluster of flowers only, showing that this length of day is very unfavorable to flowering.

WILD KIDNEY BEAN

The wild kidney bean (*Phaseolus polystachyus*), a native woodland plant, is the only representative of the genus in the Washington region. It is a scrambling twiner in the woodlands and copses which it frequents, and it flowers in July and August. The stems are herbaceous and die back to the perennial crowns each autumn. This interesting bean is a casual constituent of the flora in some situations of the upper Piedmont, as on the slopes of Bull Run Mountain and Big Cobbler, but its occurrence is usually restricted and only a careful canvass of the woodland undergrowth where it occurs will reveal its presence.

Behavior as Affected by Short Winter Days

The wild bean is very adversely affected by short days of 12½ hours or less. During the autumn of 1936 several hundred plants were grown in the greenhouse from seed that was sown October 4 and germinated October 26. These seedlings were transferred to 6-inch pots November 2, when about 3 inches tall. The young plants soon ceased to grow following the exhaustion of the reserves in the cotyledons.

The foliage on these plants consisted of a pair of simple opposite leaves above the cotyledons, succeeded by a small compound leaf with the usual three leaflets. These leaves were small, thick, crinkled, dark green in color, and never had the appearance of the thin healthy foliage of the summertime. Plants were grown in both cool (50° to 55° F.) and warm (75° to 80°) temperatures, but in neither condition did they show anything but the dwarfed and sickly growth described.

Although the plants are vigorous climbers in response to long summer days, the winter-grown plants gave no hint of elongating stems or twining habit. The plants soon passed into a state of dormancy, followed by loss of foliage, thickening of the stems, and finally death in many instances. It was obvious that the short winter days almost entirely inhibited growth in these plants, which in summertime are accustomed to become much-branched high-climbing vines.

Weak light from a 200-watt mazda bulb with reflector was supplied to a number of these plants from sunset till midnight throughout the winter. The plants quickly responded, becoming leafy twiners, but there was no indication of flowering throughout the winter, perhaps because the light periods, near 16 hours or more in length, were too long. It was evident that a very slight dosage of weak electric light at the end of the natural day sufficed to give the unhealthy winter plants a new lease on life so far as growth and twining were concerned.

Behavior in Response to Different Light Periods in Summertime

Seed of the wild bean were sown March 23, 1937. These germinated April 8 and were transferred to 3-inch pots April 23, and to buckets

May 17, with two plants in each; two buckets were included in each test. At this time the average height of the plants was 2½ to 3 inches. Two series of tests were run, one beginning May 17 and another June 4, but the results were similar. The following results with the different constant light periods were secured in the tests of June 4.

10 hours: None budded, and no twining stems were produced; height 4 inches.

12 hours: One blossom July 12 on one plant; no twining stems; height 4 inches.

12½ hours: One plant flowered July 27 and one August 11; two did not flower; slight twining; height 3 to 4 inches.

13 hours: Three plants flowered sparsely, July 16, July 24, and August 6 respectively; one never flowered; a few short runners produced; height 7 to 16 inches.

13½ hours: Three plants flowered June 23, two July 19, one July 21; runners short, the plants tending to remain bushy; height 22 to 36 inches.

14 hours: Two plants flowered July 8 and one July 27; twining habit strongly developed and plants very floriferous; height 30 to 40 inches.

14½ hours: Two plants flowered July 7 and two July 10; strong twining habit and very floriferous; height 35 to 48 inches.

Full day: Plants flowered August 15; strong twining habit and very floriferous; height 40 to 64 inches.

16 hours: Flowered August 16; strong twining habit, but sparse flowering; height 50 inches.

18 hours: Flowered October 1 at 50 inches; strong twining habit, but only a few closed flowers.

Localization Tests

Seed of the wild bean were sown March 23, and germination was evident April 8. The tiny plants were transferred to 3-inch pots April 23, and to buckets June 4. The tests began June 9, the bucket being kept inside the case (fig. 2). These plants were made to produce lateral branches by cutting back the main stem in April. When the tests began, the inside stem, receiving 10 hours of daylight each day, i. e., from 6 a. m. to 4 p. m., was 5 inches in height, and the outside stem, experiencing the full day, was 1 inch in height.

The inside stem remained very short, producing small, dark-green leafage, as in wintertime, and never budded. This portion of the plant was only 6 inches high on August 18.

The outside branch, receiving full daylight, produced buds July 21, and flowers August 2, at 60 inches. The plant was very floriferous until autumn.

BONESET

Boneset (*Eupatorium torreyanum*) is a native perennial composite of the Washington region, the herbaceous stems dying back to the rootstock each autumn. A clump was divided October 1, 1936, and the divisions were transplanted into buckets to remain out of doors throughout the winter. The various tests with these began March 29, 1937. The results were as follows:

10 hours: No buds formed; 14 sterile leafy stems, the tallest 25 inches; stems very lax, tending to bend over.

12 hours: 11 stems, none flowering; height 28 inches; stems weak, lax, arching toward the ground.

12½ hours: 9 stems, one flowering August 4 at 40 inches; stems very lax, arching.

13 hours: 13 stems, all flowering July 7 at 30 inches; stems very lax, arching.

13½ hours: Many stems, all flowering July 10 at 36 inches; stems more stiff. upright, but less erect than the controls.

14 hours: Many stems, all flowering July 27 at 35 inches; stems less erect than controls.

14½ hours: Many stems, all flowering August 4 at 33 inches; stems strictly erect.

Full day: Many stems, all flowering August 18 at 38 inches, with broad corymbose heads and erect stems.

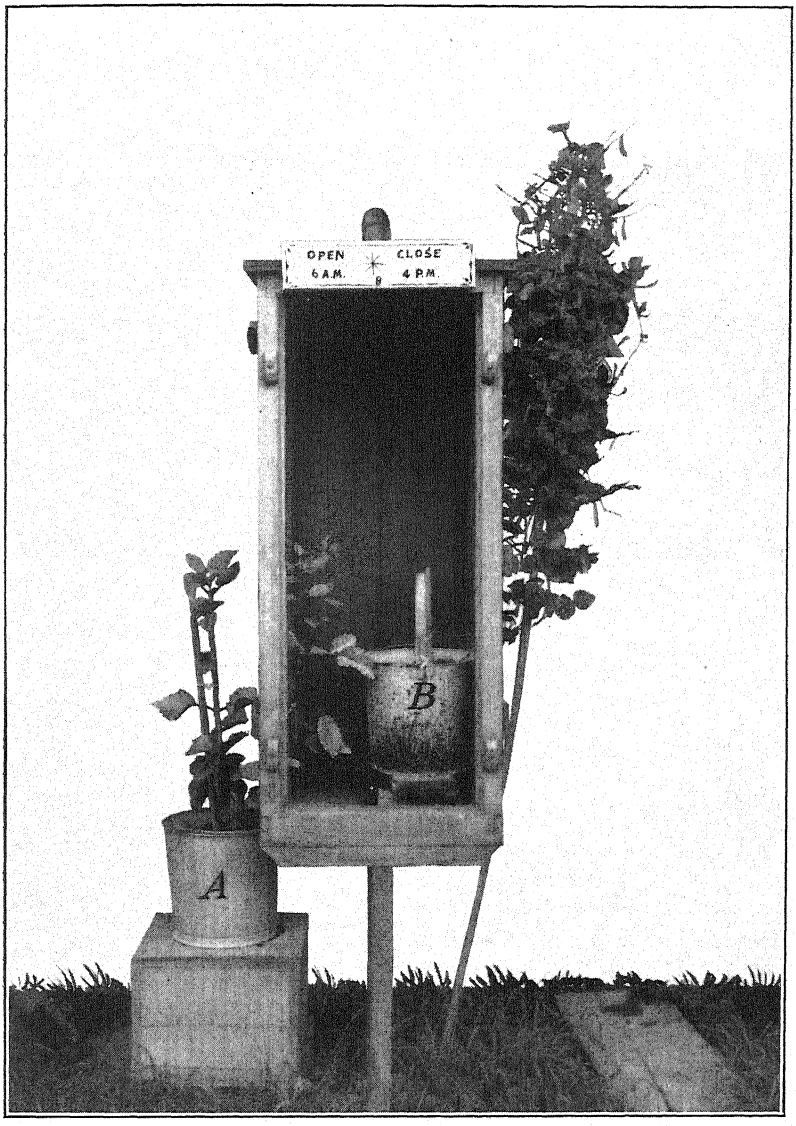


FIGURE 2.—Wild kidney bean (*Phaseolus polystachyus*) (*B*) in localization tests begun June 9. Inside portion in bucket received 10 hours of daylight each day, i. e., from 6 a. m. to 4 p. m. The branch inside grew but little beyond the original height, 5 inches, at the time the tests began, and finally died in late summer. The branch conducted outside, which received the full day, grew with great vigor, buds being visible on July 21 and open flowers on August 2. This branch was very floriferous, as shown by the pods developed. Photographed August 31. (The plant shown in *A* had no connection with the investigation reported here.)

SUGARCANE

Regulation and timely control of flowering is a matter of great importance in the breeding of sugarcane. Many varieties have shown tendencies to be very irregular in their flowering habits. In order to obtain a better knowledge of the relationship of flowering of some of the sugarcanes to length of day, the Division of Sugar Plant Investigations of the Bureau of Plant Industry arranged tests with a number of their varieties, making use of the large lightproof houses and equipment of the Division of Tobacco and Plant Nutrition.[2] Table 3 shows the growth and flowering behavior of the variety 28 N. G. 292, a form of the species *Saccharum spontaneum* found growing wild in New Guinea by E. W. Brandes, of the Division of Sugar Plant Investigations.

Stalk cuttings of this variety were made in October 1936 and were transferred first to 4-inch and then to 6-inch pots and finally to buckets. The tests were begun about May 1.

TABLE 3.—*Average length and number of stalks produced by the variety of sugarcane 28 N. G. 292 (Saccharum spontaneum) in response to different light periods*

Daily light period (hours)	Average length of stalk	Average stalks	Daily light period (hours)	Average length of stalk	Average stalks	Daily light period (hours)	Average length of stalk	Average stalks
	Inches	*Number*		*Inches*	*Number*		*Inches*	*Number*
10	36.0	14.7	14	45.2	10.6	16	37.5	12.0
12	26.0	13.4	Full day	30.7	8.3	18	41.5	12.0
13	[1] 41.7	12.7						

[1] Flower buds present.

An examination of the stalks revealed the presence of young, developing flowers in response to the 13-hour day only. From the number of stalks shown it would appear that the shorter lengths of day, represented by 10 hours and 12 hours of light each day, favored a more active vegetative condition than the 13- or 14-hour day. It would appear that the additional light from sunset has had little effect upon the behavior of the plants.

It is evident that this sugarcane has shown a very striking behavior, which places it in the intermediate class. This behavior is especially interesting since it shows an exceedingly narrow range of flowering, lying somewhere between 12 and 14 hours.

DISCUSSION

It is obvious that *Mikania scandens* finds conditions most favorable for flowering when the days are neither too short nor too long, or within a range of 12 or 12½ hours to 15 or 16 hours (figs. 3 and 4).

On the basis of its length-of-day behavior, the prediction could have been made that the northern limits of its range would be found somewhere within the latitudes of New England. As a matter of fact its range actually extends from southern Maine to Florida and Texas. It is probable that interactions between lowered temperatures, short summers, and the unfavorable influence of very long days help to define the northern limits of distribution of this plant. Length

[2] This work was under the immediate supervision of George B. Sartoris, of the Division of Sugar Plant Investigations, who has very kindly submitted the data for table 3.

of day alone, as it approaches the critical near 16 hours, retards the appearance of flowers about 5 weeks, and it is obvious that further delay can be expected where much cooler and shorter summers prevail.

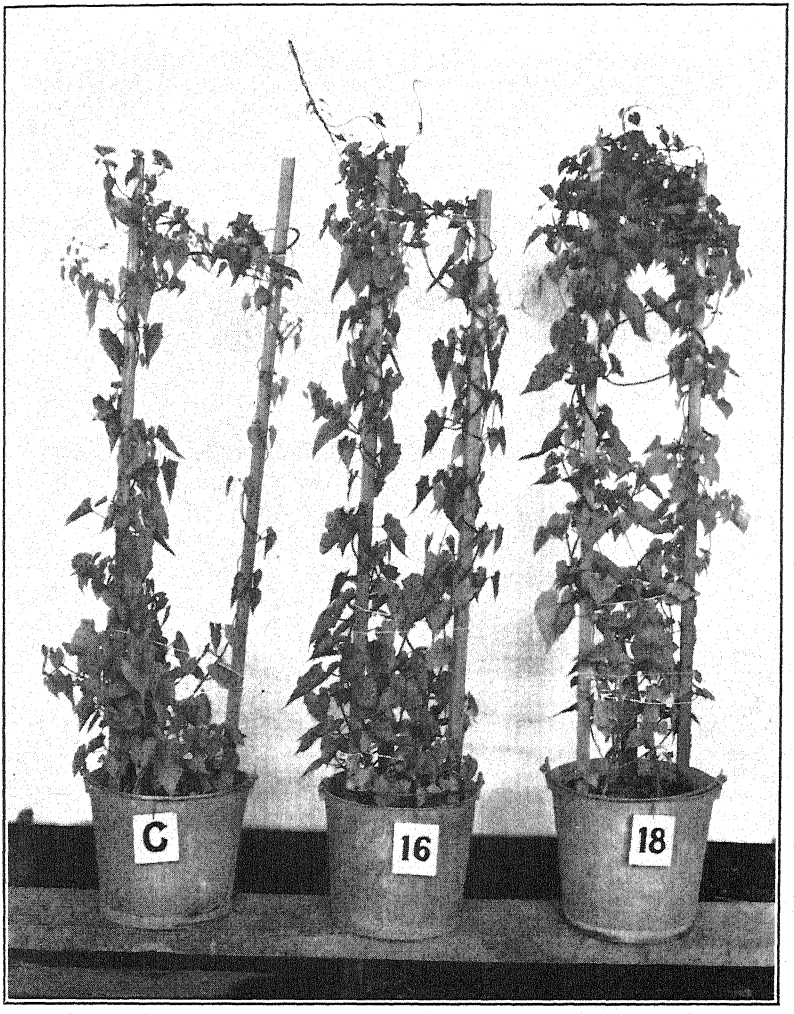


FIGURE 3.—Climbing hempweed (*Mikania scandens*) grown with indicated length of day and with full natural length of day (control, *C*). Plants in *C* flowered August 5, producing flowers in profusion, as is their normal habit. Plants receiving supplemental electric light to afford 16 hours of light produced a few flowers September 18; the plants receiving 18 hours of light never budded.

The summative effects of these factors must make successful flowering and seed production before frost a hazardous event for the species near its northern limits.

On the basis of its length-of-day behavior with respect to the lower limits of flowering, its southern distribution should depend entirely

upon length of day, for unfavorable low temperature is not a factor and the summer season is greatly extended toward the Tropics. All evidence at hand indicates that in its present genetic state the species is not adapted to the shortened days of a tropical habitat, since flowering becomes sparse and uncertain when the days are shortened much below 12 to 12½ hours.

The behavior of the plants grown from seed has been somewhat different from plants derived from old, established crowns. The former did not appear to be affected by the shortened days until a constant length of day between 12½ and 12 hours was reached; the latter when the length of day had fallen somewhere between 13½ and 13 hours.

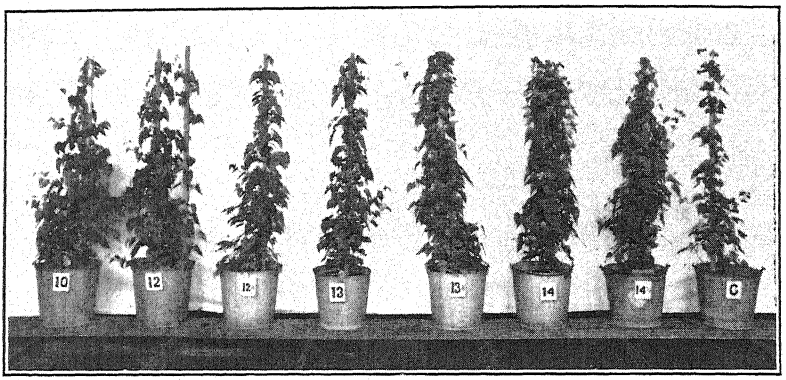


FIGURE 4.—Climbing hempweed (*Mikania scandens*). General growth behavior of plants with the various periods of natural daylight indicated on the buckets. *C* received the full daylight, serving as a control. Plants receiving 10 hours of daylight daily flowered July 24, producing only four flower clusters. The 12-hour plants, flowering July 6, the 12½-hour plants, flowering July 2, and the 13-hour plants, flowering June 28, all flowered sparsely, indicating unfavorable conditions. Copious flowering was shown when the plants received 13½, 14, and 14½ hours of light each day; flowering taking place June 25 for the 13½-hour plants and June 28 for the 14- and 14½-hour plants. The plants (*C*) receiving the full day flowered July 14, with copious flowering into September. These plants were grown from crown divisions.

The seedlings, likewise, have shown a greater tendency to flower on the higher lengths of day, since there was slight flowering in response to 16 hours of light, whereas the plants developed from rootstocks showed no tendency to flower on the 16-hour light period.

Until a careful analysis of the genetic behavior of the species throughout its range has been determined with respect to its responses to length of day, one cannot, of course, be sure that plants near the northern limits of distribution are identical in their day-length responses with those found in Florida or elsewhere in its range.

The wild kidney bean (*Phaseolus polystachyus*), like *Mikania scandens*, flowers best with an intermediate range of day lengths. Days that are too short or too long are very unfavorable to flowering and may even inhibit it entirely. The normal twining habit of the wild bean is entirely dependent upon length of day. The plants show little or no inclination toward the production of elongate branches or

the twining habit until a length of day between 13½ and 14 hours is experienced, and until the twining habit vigorously asserts itself few flowers are produced.

Vigorous flowering is not strictly correlated with the twining habit, however, since an excessively long day again tends to inhibit flowering, in comparison with the full length of day, as shown by the daily light periods of 16 and 18 hours.

On lengths of day shorter than 12½ hours the plants were of the dwarfed sterile winter type, with rather thick, dark-green rugose leaves and short stems (figs. 5 and 6). They barely survived the

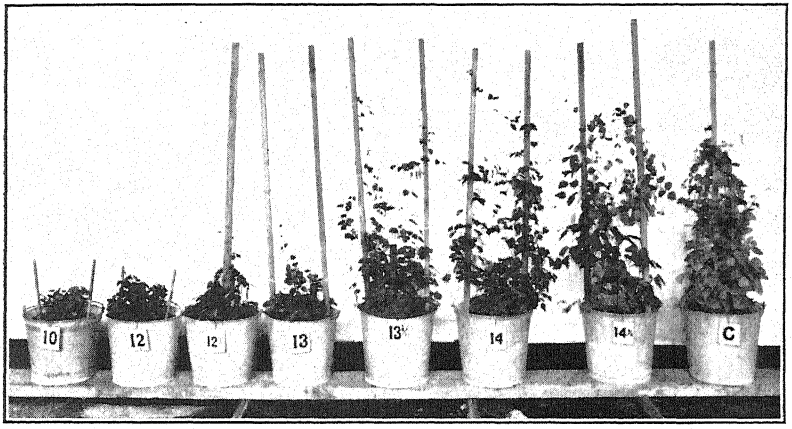


FIGURE 5.—Wild kidney bean (*Phaseolus polystachyus*). General growth behavior of plants under the various periods of daylight indicated on the buckets. *C* represents the full length of day. Plants receiving 10 hours of daylight never flowered. One flower appeared on the plants given 12 hours of light each day. Flowering was more evident with 13 hours of light, but some of the blossoms remained cleistogamic. Plants did not develop the twining habit until 13½ hours of light daily were experienced, but remained low, bushy, and dark green. Copious flowering was the rule with all light periods, including and above 13½ hours. Flowering took place on the 12½-, 13-, and 13½-hour periods from July 24 to July 28; on the 14- and 14½-hour periods, from July 6 to July 10. The controls (*C*) did not flower until August 14.

summer, and were virtually in a state of vegetative dormancy for many months. On those shorter lengths of day near the lower critical length of day for flowering, i. e., 12½ hours, an interesting response asserted itself, characterized by the production of exceptionally large flowers and pods. It is evident that under these extreme conditions leading to an almost complete suppression of twining branches, the few flowers and pods produced were assured a superabundance of available reserve material, since none of it was diverted to the support of rapidly elongating stems.

The natural distribution of the wild kidney bean from Connecticut to Florida is in agreement with its day-length responses and limitations as revealed in the tests. Its inability to flower on the shorter lengths of day and its sparse and delayed flowering when the days are considerably lengthened would preclude a successful invasion into tropical latitudes on the one hand or a far northern distribution on the other, if it is assumed that the genetic constitution is like that which the tests

revealed. The plant probably finds its best adaptation near the middle ground of its range, rather than at the critical extremes where conditions are always more hazardous for any species.

The most striking features of the behavior of *Eupatorium torreyanum* are the development of sterile stems on lengths of day of 12½ hours or

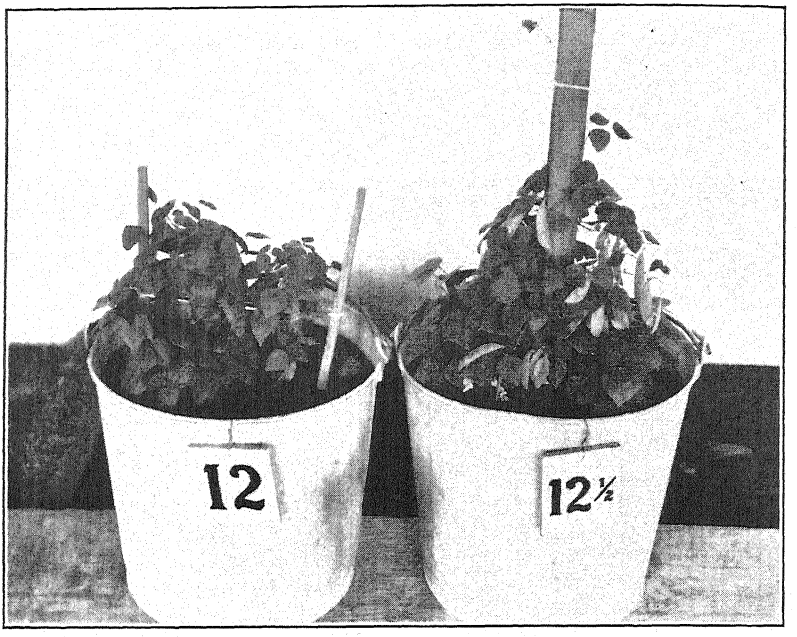


FIGURE 6.—Wild kidney bean (*Phaseolus polystachyus*) grown under lengths of day indicated. Plants receiving 12 hours of light daily never flowered. One of the plants receiving 12½ hours of light flowered July 27; the second never budded. The plants were practically bushy in habit of growth except for one short runner, and produced exceptionally large beans for the species, some of the pods measuring 5.8 cm in length from the tip of the beak to the calyx, with a maximum width of 11 mm. The wild bean makes little growth and can neither twine nor flower when the days are much below 12½ hours in length.

less and the development of very weak, lax, or arching stems until the longest light periods have been reached. The shortest day lengths have strictly inhibited flowering. The 12½-hour day, which appears to be near the critical length for the initiation of flowering as the days are lengthened, greatly delayed flowering as compared with the most favorable day lengths of 13 to 13½ hours (fig. 7). It is obvious that at the higher lengths of day, near 14 hours, flowering is again very noticeably delayed. From the data at hand it would appear that this boneset is not adapted to the extremely long days of northern latitudes nor to the extremely short days of tropical regions, and its natural distribution conforms with these limitations of its day-length behavior, ranging as it does from Pennsylvania to Florida and Texas.

INTERMEDIATE BEHAVIOR AND THE ADAPTATION OF PLANTS

The permanent colonization of plants, whether natural or through human agency, depends upon the inherent adaptive powers of the

species. Since length of day has been shown to be a very potent factor in the control of flowering, it is obvious that the length-of-day responses play a very important part in permanent colonization in any region. The position of the critical length of day for flowering and its sharpness or narrowness may very definitely determine in what latitudes a plant will survive and produce seed before frost. Short-day plants are adapted to distribution in lower latitudes, while long-day plants require the long summer days of higher latitudes. Just how far north short-day plants can succeed depends upon how far into the long-day region the critical length of day is extended, and just how far south long-day plants will spread depends upon how far down toward the shorter days the critical length of day is situated.

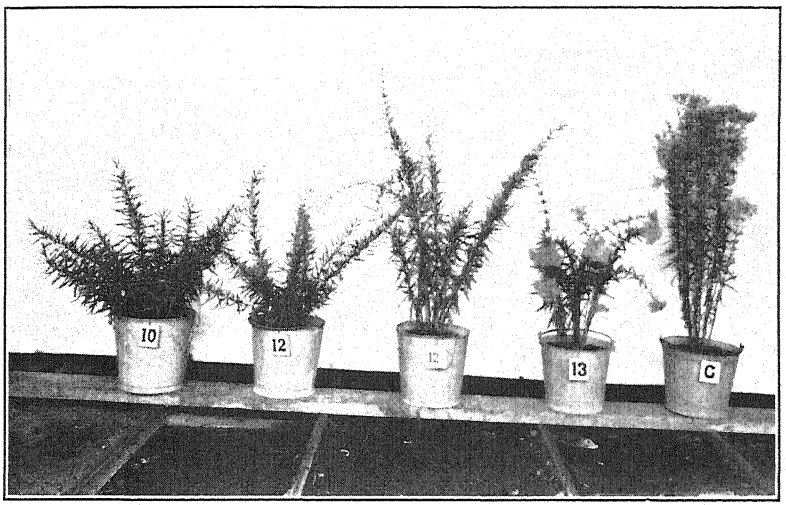


FIGURE 7.—Boneset (*Eupatorium torreyanum*) plants grown under the various lengths of day indicated. The control plant (*C*) was grown under the full length of day. Stems were lax and unable to flower on shorter day lengths. A single stem flowered on plant receiving 12½ hours of light; flowering was profuse with 13 hours of light daily, but stems were weak and lax. Stems stiffly erect on control (*C*). The dates of first flowering follow: 12½-hour day, August 4 at a height of 40 inches; 13-hour day, July 7, at a height of 30 inches; full day, August 18, at a height of 42 inches.

Unlike the long-day and the short-day groups, plants of intermediate habit have their limitations determined by two critical points, a maximum in the direction of long days and a minimum in the direction of short days. If the minimum in the direction of the short days is near 13 hours, as in the case of the wild kidney bean, it is evident that the plant cannot flower in latitudes much below 20°, where the longest days of the year scarcely reach this critical length of day.

Eupatorium torreyanum would not flower in the Tropics in latitudes much below 10°, since the minimum critical length of day for this plant is near 12½ hours. On the basis of their length-of-day requirements, *Mikania scandens*, *Phaseolus polystachyus*, and *Eupatorium torreyanum* are not fitted to flower and to reproduce seed near the Equator, where the length of day approaches a constant of about 12 hours throughout the year.

The sugarcane variety 28 N. G. 292 has shown a definite flowering impulse in response to a daily light duration of 13 hours, and a vegetative condition with 12 and 14 hours. It is possible, however, that a length of day approaching 12½ hours and one somewhat longer than 13 hours would also have been favorable to flowering. On this basis this variety can be expected to flower almost on the Equator or at least in tropical latitudes ranging from 4° to 5° up to 20° or more, and this appears to be its normal behavior. This sugarcane has flowered at Guayama, Puerto Rico, practically on the eighteenth parallel, November 9. When this sugarcane is grown in Louisiana near the latitude of 30° N., flowering becomes irregular and may not take place until December. This behavior would indicate that this cane does not find length-of-day conditions very favorable to flowering much outside the Tropics, the northern and southern boundaries of which are 23½° from the Equator. If this behavior is an inherent condition of this variety, as indicated by the very narrow flowering range in the tests, flowering could not be expected in northern latitudes.

In the narrow responses of this cane one sees a highly specialized length-of-day behavior that has not previously been encountered. The very narrow restriction of its flowering responses, however, fits it admirably for naturalization in the Tropics alone, where it has been found at home.

In the case of the sugarcane it must be emphasized that the results presented were secured from a single test. It is thought, however, that the finality of the results of this test indicates the behavior of the variety under the conditions.

The wild kidney bean, *Mikania scandens*, and *Eupatorium torreyanum* are well adapted to middle latitudes lying roughly between 25° and 45°. The north and south range of such intermediate plants depends upon the upper and lower limits of their day-length responses. If these are far apart the range of the plants will be wide; if they are close together the range, as in the case of the sugarcane 28 N. G. 292, will be restricted. In the case of this cane, the capacity to flower lies somewhere within a range in length of day of only 2 hours, and the low upper limit at which flowering fails keeps the plant within the Tropics. While such plants have not yet been found, it is possible that intermediate plants quite as narrow in their length-of-day requirements for flowering will be found to exist in far northern latitudes. Such plants, like those in the long-day class, flowering in response only to very long days, could adapt themselves only to far northern latitudes. On this basis it is possible that some plants require continuous light for their best development.

SUMMARY

A number of plants of intermediate behavior have been found. They constitute a group of plants whose flowering is favored by lengths of day neither too short nor too long. On either side of this optimum range, flowering may cease entirely or be delayed or less profuse.

Mikania scandens, *Phaseolus polystachyus*, *Eupatorium torreyanum*, all native wild plants of Washington, D. C., and the wild New Guinea sugarcane 28 N. G. 292 show the intermediate flowering behavior.

Mikania scandens flowers very poorly in response to lengths of day that are 12 hours long or less, and flowering has practically ceased when the days have been lengthened to 16 hours or more.

Phaseolus polystachyus is near its flowering limit when the days are reduced to 12½ hours, and flowering is again checked or delayed when the days have been lengthened to 16 hours or more.

Eupatorium torreyanum ceases to flower when the length of day has been reduced to about 12½ hours or less. Experiments indicate that the plants flower most readily with 13 to 14 hours of daylight, and show a delay in flowering when the daylight periods exceed these values.

The wild New Guinea sugarcane 28 N. G. 292 of the species *Saccharum spontaneum* L. has shown the narrowest flowering response of any plant yet studied. Tests with daily light periods ranging, at 1-hour intervals, from 10 to 14 hours, and with the full length of day, at Washington, D. C., revealed that flower buds were formed only when the plants were afforded 13 hours of light each day. The flowering range for this species, therefore, lies somewhere between 12 and 14 hours of daylight.

U. S. GOVERNMENT PRINTING OFFICE: 1938

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 57 WASHINGTON, D. C., DECEMBER 1, 1938 NO. 11

STAGONOSPORA LEAF SPOT AND ROOT ROT OF FORAGE LEGUMES[1]

By FRED REUEL JONES and J. L. WEIMER, *senior pathologists, Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*[2]

INTRODUCTION

A root rot of alfalfa (*Medicago sativa* L.) not previously described was found almost simultaneously at Riverside, Calif., and Madison, Wis. The causal agent has been shown to be a species of *Stagonospora* known previously only as a leaf-spotting fungus of several legumes. Since no leaf spot was found associated with the root rot, at least in California, a study was made to determine definitely the identity of this fungus with similar fungi on other closely related plants. In these investigations all available herbarium material of these fungi on all of the forage legumes in the genera *Medicago*, *Melilotus*, and *Trifolium* has been examined and cultural studies of living material have been made as far as possible.

An ascigerous stage belonging in the genus *Leptosphaeria* has been found on certain host species but not on all of them. For this reason the writers have chosen to designate the disease on all of the hosts as stagonospora leaf spot and root rot. A new combination is suggested for the *Stagonospora* on *Trifolium pratense*.

THE DISEASE

In the production of disease of possible economic importance within the observation of the writers, the species of *Stagonospora* with which this paper is chiefly concerned merits attention as a cause of a root rot of alfalfa and of a leaf spot and stem blight of sweetclover. The fungus has been found producing a seemingly unimportant leaf spot on other host species, and has been cultured from them, the complete list being as follows: *Medicago sativa* L., *M. lupulina* L., *M. arabica* Huds., *Melilotus alba* Desr., *M. officinalis* (L.) Lam., *M. dentata* (Waldst. and Kit.) Pers., *Trifolium repens* L., *T. hybridum* L. It has been seen in herbarium collections on *Trifolium alpestre* L. The leaf spot on all of these hosts is so similar that the description of it on alfalfa applies adequately to all.

[1] Received for publication July 22, 1938; issued December 1938. Cooperative investigations of the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture; the Department of Plant Pathology, Wisconsin Agricultural Experiment Station; and the Division of Agronomy, California Agricultural Experiment Station.

[2] The authors gratefully acknowledge the contribution of valuable mycological collections from the Herbarium of the University of Wisconsin, the mycological collections of the Bureau of Plant Industry, U. S. Department of Agriculture, the Farlow Herbarium, and J. Dearness. They are also especially indebted to J. J. Davis, of the University of Wisconsin, and to J. A. Stevenson and Edith K. Cash, of the Division of Mycology and Disease Survey, Bureau of Plant Industry, for the critical reading of the taxonomic portion of the manuscript and other assistance.

On alfalfa the leaf spot is a light buff, pale olive buff, to almost white (fig. 1, *A*). Many of the spots have a darker brown border—bister to sepia of Ridgway (*35*)[3]—between the gray center and the green healthy tissue. In some spots there are other, darker brown,

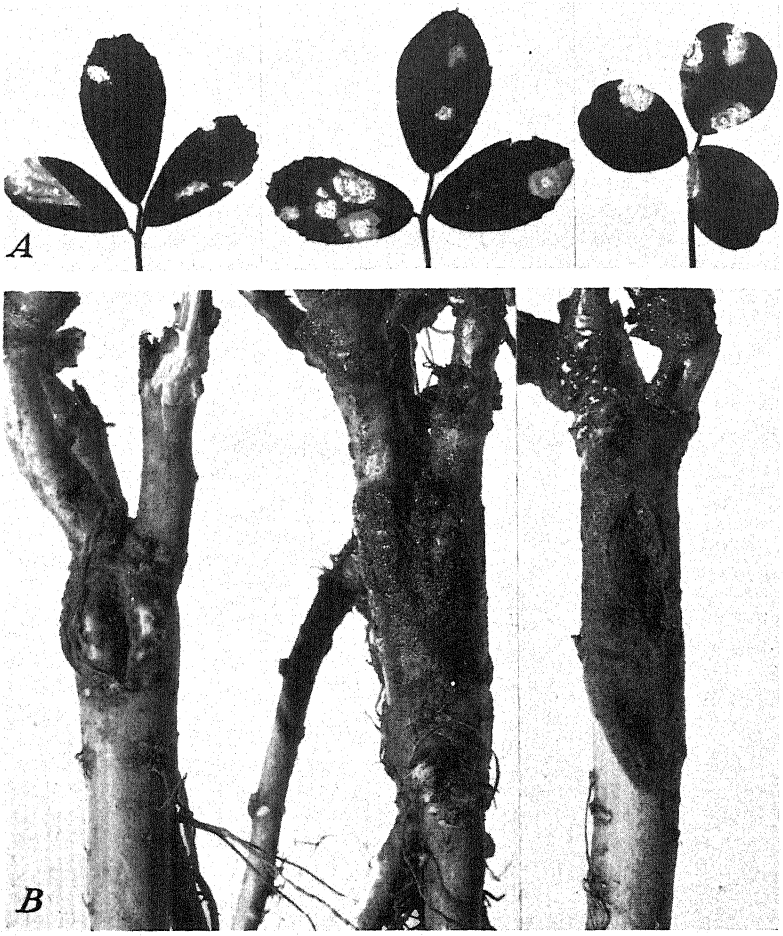


FIGURE 1.—Leaf spot and root rot of alfalfa produced by artificial inoculation with *Stagonospora meliloti* at Riverside, Calif. *A*, Leaf spot of alfalfa seedlings produced by inoculation. × 1¼. *B*, Root and crown rot of alfalfa produced by inoculation with *S. meliloti* from alfalfa. The wound on the uninoculated plant at the left is completely healed. Inoculation made August 4, 1931; photographed June 9, 1932. × about 1¼.

more or less concentric narrow bands in the central gray portion of the spot. The margins of the spots are sometimes water-soaked in appearance. In the light central portion are numerous light-brown to nearly black pycnidia. Pycnidia are sometimes developed near the margin of the spot when the foliage is kept at high humidity.

[3] Italic numbers in parentheses refer to Literature Cited, p. 810.

The spots range from 1 mm to 1 cm in diameter, or they may involve nearly the entire leaflet.

Stem blight was produced on alfalfa, both at Riverside and at Madison, but was not observed in the field. Ashy lesions bearing pycnidia often girdled the stems, causing the death of the shoot. Petioles were likewise attacked.

As a root rot of alfalfa, the disease occurred at Riverside only on the upper part of the taproot and on the crown branches (fig. 1, *B*). The surface of the lesion is dark brown to black in color, usually smooth at first, but becoming roughened as the outer layers dry and crack. Beneath the surface the tissues are reddish brown to almost black. They are moist at first but dry out later, forming a dry rot that may involve a considerable part of the root or crown. The taproot may be severed by a lesion. The disease progresses slowly, requiring several months in which to rot off a medium-sized alfalfa root. A characteristic reddish color is often present in the decaying wood rays through which the disease makes its way toward the center of the root. No new buds are formed above the lesion, and the plant eventually dies. Since the disease progresses slowly, secondary invaders enter the diseased tissue and undoubtedly contribute largely to the destruction of the plant.

As a root rot the disease was first found at Madison in the overgrowths of grafted plants transplanted to the field. Here the lesions strongly resembled crown rot caused by *Phytomonas insidiosa* McCulloch, from which the stagonospora root rot was first distinguished by microscopic examination. Later the stagonospora rot was found in plants inoculated with *P. insidiosa* for the purpose of testing their resistance. Finally *Stagonospora* was discovered causing a crown rot in a small percentage of plants in an alfalfa field. The most characteristic root rot lesions are wedge-shaped discolorations extending from the bark toward the center of the root. The crown rot is distinguished in the field only with difficulty, and thus an estimate of its importance in comparison with other diseases is not easily made.

On *Melilotus* spp. the leaf spot has usually been found most abundantly early in spring and late in the fall, on foliage produced in cool weather. However, on a variety of sweetclover with fine stems the leaf spot has been abundant in midsummer as well.

The fungus invades stems extensively on occasion, though for the most part so late in the fall that the damage is probably slight. Once the tops of young tender shoots in a dense growth were found killed by the fungus, which was fruiting abundantly. Only once has the *Stagonospora* stage been found fruiting abundantly on flowering stems, and in this case the plants had been checked by drought. Here the lesions resembled so closely those of a species of *Ascochyta* occurring on this plant that the fungus was recognized only after microscopic examination. In early autumn the bases of stems of plants of *Melilotus alba* are often invaded extensively by the fungus in a manner described later, with little external evidence beyond a paler green color and a slight browning of the interior tissue until the *Phoma* stage of the fungus begins to develop, when the color changes to a golden brown and the surface becomes finely dotted with the deeply seated fruiting bodies.

A root rot of sweetclover has been produced readily by inoculation, but has been found only once in the field. Lesions may be black in early stages, but when extensive are usually ash-colored and dry.

PATHOLOGICAL HISTOLOGY

At Madison the stagonospora root rot was first recognized and distinguished from bacterial wilt of alfalfa by the discovery in diseased tissue of blue-staining fungus threads where blue-staining bacteria were expected after the application of the Gram stain (*33, p. 17*). After some exploration it was found that the fungus mycelium has this unusual and most useful staining reaction only when growing in certain situations. In roots and in large stems on which the fungus has not fruited it stains well; in leaves and in culture media the small fragments of fungus thread that have been stained are so few that for practical purposes it may be said not to stain at all in those situations. Spores in pycnidia often stain brilliantly, both on the host plant and in culture. The staining of a fungus in its host plant with the Gram stain, as described above, has not been previously recorded so far as the writers are aware. Roots in which the stain revealed the fungus were cultured on many occasions, and in no instance did the fungus fail to grow. Finally the stain was used as a rapid and, it is believed, a reliable test for the invasion of the fungus both in field material and in inoculated roots when the lesions were of doubtful character.

The fungus appears to enter roots of alfalfa and sweetclover only as a wound parasite. The mycelium is easily stained and traced between the parenchymatous ray cells in approximately the same region often occupied by the bacteria causing wilt. However, the fungus develops more extensively between the larger cells at the center of the rays rather than between the smaller cells at the margins of the rays, where the bacteria are more frequently found, and thus it does not approach or enter the vessels. Mycelial strands are often found far in advance of discoloration in invaded tissue (fig. 2, *B*). Sometimes the older mycelium bears small knobs resembling haustoria, but their penetration of the host cell walls against which they are pressed has not been satisfactorily demonstrated. Overgrowths of any kind, where cells and intercellular spaces are large, appear to be particularly favorable regions for invasion (fig. 2, *A*).

PATHOGENICITY OF THE FUNGUS

INOCULATION EXPERIMENTS AT RIVERSIDE

Isolations were made from several diseased plants, and cultures of several species of fungi were obtained. On April 14, 1931, 5 of these species, including the species of *Stagonospora*, were each inoculated into 10 healthy alfalfa plants growing out of doors in 16-gallon garbage cans, and 10 plants were held as controls. The plants were about a year old, and the roots measured 8 to 10 mm in diameter near the wound. Hyphae from agar cultures were inserted into wounds made in the taproots just below the crown, while the controls were wounded but not inoculated. On June 9, 1932, the plants were removed from the soil and examined. Six of the plants inoculated with *Stagonospora*

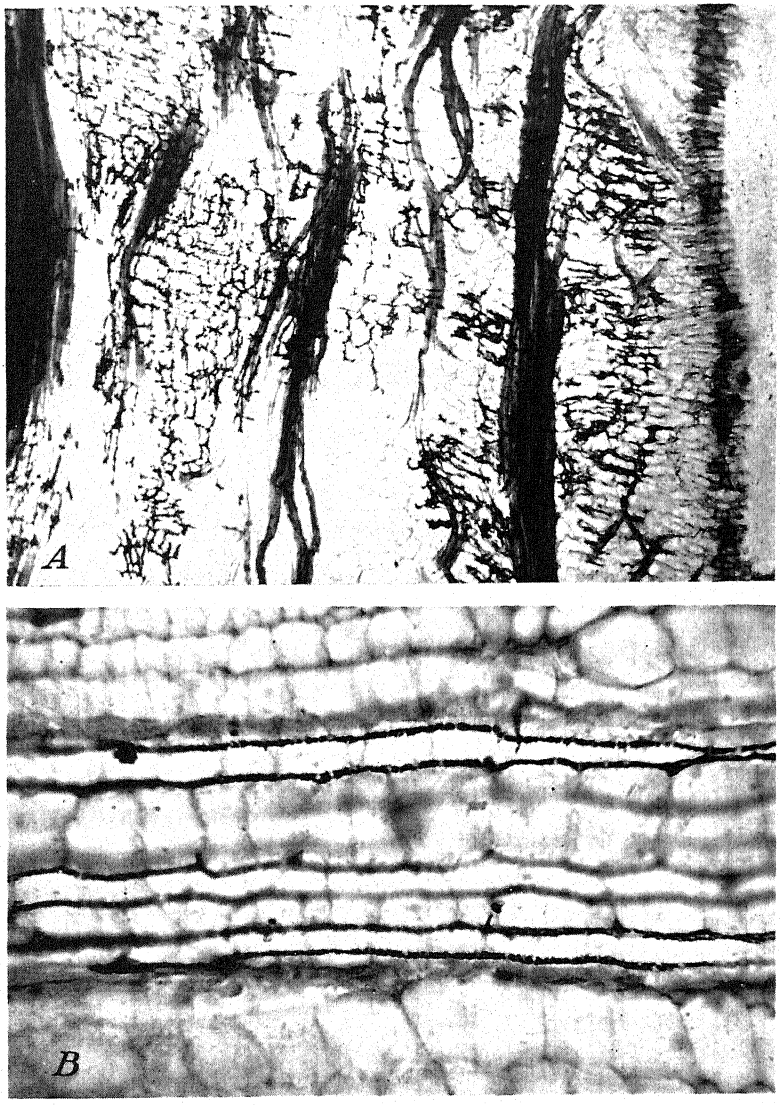


FIGURE 2.—Mycelium of *Stagonospora meliloti* stained in host tissue. *A*, Mycelium in a longitudinal section of the phloem of a grafted alfalfa plant. Slight discoloration of the living tissue indicated this diseased condition. The fine dark lines represent deeply stained intercellular mycelium. From natural infection. *B*, Mycelial strands of the same fungus passing through a wood ray of *Melilotus alba*, far in advance of discoloration. From artificial inoculation. × 450.

were more or less decayed about the wound. The other roots, including the controls and those inoculated with the other fungi, were healthy. Figure 1, *B*, shows two of the infected plants and one of the controls.

A second set of inoculations was made in January 1934. Alfalfa plants about a year old were inoculated and set in cans, which were held in soil-temperature tanks at five different temperatures, namely, 36°, 32°, 28°, 25.5°, and 19°–21° C. Ten plants, inoculated by inserting bits of hyphae from an agar culture into wounds, were held at each temperature. All of the wounds were covered with rubber electric tape. The temperature usually did not vary over 1° except in the 19°–21° tank, which varied as much as 2°. The experiment was discontinued on April 18, 1934. At no time were there any top symptoms that suggested root infection. When the roots were examined it was found that there were 0, 3, 10, 6, and 9 plants infected at 19°–21°, 25.5°, 28°, 32°, and 36° respectively. There was no infection in any of the controls. The lesions were all small, and it was evident that the experiment should have been continued several months longer. The fungus had spread into the tissues and formed dark-reddish islands or streaks, such as are characteristic of the decay in the deeper tissues of naturally infected plants. Isolations were made from a number of plants, with the following results: The fungus was recovered from eight of the plants infected at 36°, from four at 32°, and from seven at 28°.

On June 12, 1934, 7 lots of 25 healthy 1-year-old plants were inoculated with 7 isolates of the fungus respectively. The hyphae from agar slants were inserted beneath the bark through wounds which were then closed by being wrapped tightly with raffia. Twenty-five controls were prepared by wounding and wrapping. The plants were then set in an isolated plot of ground on which alfalfa had not been grown for several years, if ever. Thirty-five of the plants did not survive the transplanting. The number of healthy and diseased plants alive on October 11, 1934, as well as the percentage of infection, is shown in table 1. Of the inoculated plants alive on October 11, 1934, 49.3 percent were infected. Most of the lesions were small but were sufficiently far advanced to show their true nature. None of the controls was infected.

TABLE 1.—*Percentage of living plants diseased on October 11, 1934, out of 25 healthy 1-year-old alfalfa plants inoculated with different isolates of Stagonospora meliloti on June 12, 1934, at Riverside, Calif.*

Source of fungus	Healthy plants	Diseased plants	Infection
	Number	*Number*	*Percent*
(1) Natural infection, Riverside	16	5	23.81
(2) Do	13	3	18.75
(3) Artificial inoculation with No. 2	4	19	82.61
(4) Same as No. 3, but another isolation	11	11	50.00
(5) Same as 3 and 4, but another isolation	13	8	38.10
(6) Natural infection, Madison [1]	8	8	50.00
(7) Do.[1]	7	16	69.57
(8) Control	23	0	.00

[1] Cultures supplied by F. R. Jones.

Although there was no field evidence of the parasitism of this fungus on leaves, inoculations of foliage were made. On March 18, 1935, two 6-inch pots of alfalfa seedlings about 6 inches tall, grown from seed in the greenhouse and free from leaf diseases, were sprayed with a spore suspension. The plants were then held with controls for 3 days in an inoculation chamber where the humidity was high.

The time required for infection to appear was not determined, but on April 4 numerous more or less circular or rarely V-shaped spots were present on the leaves of many of the inoculated plants. A fungus which proved to be identical with that used in inoculation was isolated from several of the leaf lesions.

INOCULATION EXPERIMENTS AT MADISON

The inoculation trials at Madison were designed chiefly to determine whether the *Stagonospora* stage of the fungus passes readily from one host species to another. Foliage and root inoculations were made, chiefly on alfalfa and sweetclover with spores from cultures.

Foliage inoculations were largely unsuccessful. However, alfalfa was infected with cultures from alfalfa, and sweetclover was infected with some cultures from sweetclover. *Trifolium repens* was not infected with spores from any source. In the cross-inoculation trials the only success was a slight infection of white sweetclover foliage with one culture from alfalfa. Cultures from *Medicago lupulina* and *M. arabica* were not available when most of these tests were made, and therefore these have not been tested on foliage. In these inoculations, as in those at Riverside, it was at least 2 weeks after inoculation before spots appeared on the leaves. This long incubation period was unexpected, since the leaf spot occurs in the spring on leaves that can hardly be 2 weeks old. Moreover, with alfalfa the Peruvian and non-winter-hardy varieties were infected more readily than hardy Turkistan plants. Experience with these foliage inoculations suggested two things: (1) Differences in varietal susceptibility within a species might be greater than difference in susceptibility between species; (2) in the field another spore form in addition to conidia might be responsible for infection. The successful search for the perfect stage was in part inspired by the latter possibility. Since the perfect stage has been found in sufficient quantity for inoculation work only on sweetclover, few cross inoculations with it on foliage have been attempted. Thus far success has been obtained only in infecting foliage of sweetclover. No inoculations have been attempted with the *Phoma* stage.

Root inoculations with the fungus from various host sources were tried in the hope that these would furnish an easier test of the ability of the fungus to pass from one host to another. Sweetclover roots were used more extensively than those of alfalfa, since they could be stored more readily for use during the winter and could be sectioned more easily when microscopic examination of the lesions was necessary. As in the previously cited experiments at Riverside, rapid infection could be obtained only at moderately high temperatures, and for most of the work a soil temperature maintained at about 24° C. in tanks was chosen. A typical result is given in table 2. In this experiment the inoculum was inserted beneath the surface of the thick phloem of roots about half an inch in diameter. The

control plants were wounded in the same manner as those inoculated. At the end of the experiment the plants invaded by the fungus to the center of the woody part of the root were usually so badly discolored that no microscopic examination was necessary to determine the extent of the fungus invasion. In other cases, the extent of fungus penetration was determined by staining microscopic preparations with the Gram stain.

TABLE 2.—*Infection in 3 roots of Melilotus alba, inoculated with cultures of Stagonospora meliloti from different host sources, after 40 days at 24° C.*

Culture No.	Host source	Number of plants			
		Uninfected	Phloem invasion		Wood invasion
			Slight	Extensive	
1287	*Melilotus alba*				3
1403	do			1	2
1260	*Medicago arabica*	2	1		
1347	*M. lupulina*	3			
1226	*M. sativa*	3			
1322	do	1		2	
1195	*Trifolium hybridum*				3
1232	do			3	
1184	*T. repens*	2			1
	Controls	6			

From the data given in table 2, supplemented with data from several similar trials, it appears that not all isolates from *Melilotus* are equally effective in infecting *Melilotus*. A similar situation was found with isolates from alfalfa, as shown in the results at Riverside given in table 1. While isolates from *Melilotus* as a group infect *Melilotus* more severely than any similar group yet obtained from the other host species, yet in the other host groups of isolates that have been made large enough to be significant some isolate has been found capable of infecting *Melilotus* to a degree approximating that of the less pathogenic isolates from *Melilotus* itself. Thus this method of inoculation has not served to disintuish any possible host-limited biological races in this species, though it has not been used sufficiently to lead safely to the conclusion that none exists. A thorough exploration of the extent to which and the conditions under which the fungus may pass from one host to another was considered to be beyond the province of this investigation.

MORPHOLOGY AND DEVELOPMENT OF THE FUNGUS ON HOST PLANTS

SUCCESSION OF THE THREE FRUITING FORMS ON HOST PLANTS

The fungus has been found producing on sweetclover three fruiting structures, of which the commonest and best known belongs in the genus *Stagonospora*. In addition to this, in the autumn a second fruiting form belonging in the genus *Phoma* develops on plants which are late in maturing or which continue producing flowers after the main crop of seed is set. The *Phoma* pycnidia appear around lesions in which the *Stagonospora* pycnidia developed earlier, usually near the bases of stems but sometimes upon small branches. This second or *Phoma* form appears to be distinct in character from the earlier or *Stagonospora*

form, and not a variation such as occurs in the *Stagonospora* on narcissus (*12*). The third form, belonging in the genus *Leptosphaeria*, appears on dead stems the following spring and continues to develop during the entire summer and autumn if these stems are well preserved.

The extent to which the three spore forms function in infection and in the perpetuation of the fungus is not clearly apparent from observation and studies. The *Phoma*-like spore form, found thus far only on second-year stems of *Melilotus* spp., does not appear to be suitably situated either in place or in time to serve as inoculum. In fact, no clear demonstration of its ability to infect has yet been made. The *Stagonospora* form, developing abundantly on dead first-year stems of sweetclover, is in excellent position to initiate the spring infection on that host. This may occur very early in the spring—it was found April 28 at Madison, Wis., in 1938—and at this time the pycnidia of this conidial form were almost always found on the overwintered stems standing in the midst of the young growth. The ascigerous stage was also found occasionally on these stems, but the ascospores were not mature. However, mature ascospores had been found a month earlier on first-year stems in a few locations; thus, if these served for inoculum they must have been wind-carried over some distance. That this may have been the case is indicated by the presence of the leaf spot on occasional isolated plants near which the fungus was not found on dead stems. After the middle of May the ascospores were found maturing abundantly. However, the infection of the upper foliage on rapidly growing plants rarely takes place unless these plants are spaced in rows. Thus the leaf spot, which seems from spring development capable of doing great damage to sweetclover, becomes less conspicuous during the period of rapid growth and may become abundant on the rosettes of leaves in late autumn.

On alfalfa no abundant early spring development of the leaf spot has been noted, even when dead stems of the previous year bearing pycnidia are present. In late spring the leaf spot has been found moderately abundant on plants isolated in thin stands or in rows, but not in thick stands. The ascigerous stage has been found sparingly with the conidial stage on occasional overwintered stems. Here, as with sweetclover, the relative abundance of the conidial stage can be taken as evidence that it serves chiefly as inoculum in the spring, while the ascigerous stage serves for widespread though sparse distribution of the fungus.

On the other host plants the fungus has not been observed as early in the spring as on sweetclover. Collections of the leaf spot have been made chiefly in the summer.

Early in the study of this fungus it became clear that not only was the chief specific character, spore size, highly variable, but that even the chief generic character, spore septation, was often absent not only in spores from cultures but also in those from the host. In 1935, herbarium collections were assembled and examined in the search for a species character of the fruiting structure as dependable as the staining reaction of the mycelium had proved in the identification of the fungus in infected roots. This character was found in the shape of the pycnidial neck or rostrum. When examined in paraffin sections of the fruiting structure, this rostrum was found to have a central canal narrower at the base than at the apex (fig. 3, *A*). The height of this rostrum varies in pycnidia in a single leaf spot, and when it is

short its flaring character is not prominent. However, pycnidia are usually abundant, and rarely need one search far to find a rostrum of the character illustrated. Pycnidia of other fungi on these hosts have been examined without a single rostrum of this characteristic form being found. Several of the descriptions of this fungus have mentioned the presence of a rostrum, but none has noted its peculiar shape, probably because this is readily observed only in well-prepared sections. Figure 3, *B*, illustrates a pycnidium of a closely related species, *Stagonospora recedens*, on *Trifolium pratense*, which is dis-

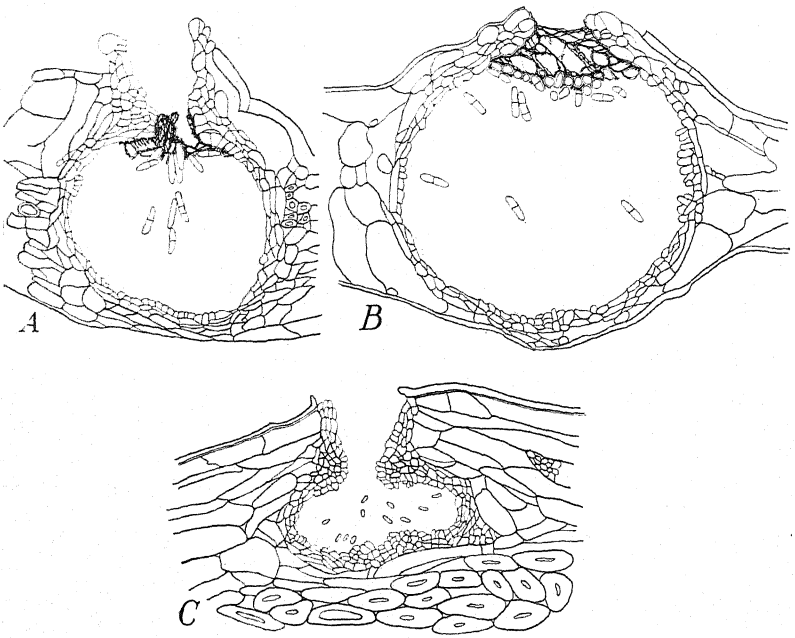


FIGURE 3.—Comparison of the two kinds of pycnidia of *Stagonospora meliloti* (Lasch) Petr. with that of *S. recedens* (C. Massalongo) n. comb.: *A*, A small pycnidium of *S. meliloti*, drawn from the Lucan collection for Fungi Columbiani No. 1335 and North American Fungi No. 2959; *B*, *S. recedens*, drawn from *Phleospora trifolii* Cav. var. *recedens* C. Massalongo in Krieger, Fungi Saxonici No. 1750; *C*, the *Phoma*-like stage of *S. meliloti*, drawn from a collection of the base of large stems of *Melilotus alba*. The thin parenchymatous rostrum with greater diameter at the apex, shown in *A*, is characteristic of *S. meliloti* but is absent from *S. recedens* as shown in *B*, though a short rostrum with isodiametric opening is sometimes found in this species. The characteristic rostrum occurs in the *Phoma*like stage of *S. meliloti*, as shown in *C*, when the pycnidia arise deeply in the tissue of large stems, but is often absent from pycnidia on small branches. × 230.

tinguished sharply from *S. meliloti* by the absence of the characteristic beak.

When the *Phoma* stage was found, the pycnidia were examined for the presence of this character. The pycnidium of this stage is developed in the parenchymatous exterior tissue of the stem, and where this is thin, on small branches, the neck of many of the pycnidia is so short that it has no noteworthy character. Wherever the origin of the pycnidium is deep enough to permit the development of the neck, it is like that of the pycnidia producing the larger spores (fig. 3, *C*).

SPORE MEASUREMENTS ON HOST PLANTS

Since the measurements of the *Stagonospora* type of spores in different collections were found to differ considerably, it appeared at first that these differences might be correlated with the host on which the fungus was found. To test this hypothesis spores were measured from seven collections from each of three of the principal hosts. The mean spore length of 50 spores taken at random was chosen as the datum most likely to show differences. It was found that on *Melilotus alba* the mean spore length ranged from 15.8μ to 19.3μ; on *Medicago sativa*, from 15μ to 19.5μ; on *Trifolium repens*, from 13.7μ to 17.7μ. These data show first of all a large variation in spore length among the collections on each host species. The spores of the fungus on *T. repens* appear shorter than those from the two other host species; yet if a single collection giving the lowest mean is excluded—a collection typical in all other respects—the range of measurements lies inside that of the measurement of spore length on alfalfa. In other words, the spore measurements on *T. repens* vary less than on the other hosts. Thus the fungus is not distinguished by consistent differences in spore size on these three hosts nor on any of the other hosts from which collections were examined later.

MORPHOLOGY AND DEVELOPMENT OF THE FUNGUS IN CULTURE

The general appearance of the fungus in culture has varied so much with age and origin that a simple precise description is impossible. When the isolate is derived from ascospores discharged upon malt agar the mycelial development from each group of spores is slow, with an early development of pycnidia. Conidia of both kinds planted in agar give cultures of much the same character. Pycnidia are formed at or near the center of growth. When this mycelial growth is transferred to agar slants, the transfer usually becomes a thick mat from which the mycelium spreads slowly from the central fungus mound. The aerial mycelium is scanty and white, yellowish, or gray in color. Isolates from mycelium in living tissue usually develop more rapidly on agar, with more mycelium and less fruiting, than cultures from spores. After several mycelial transfers the fungus may cease to fruit. The fruiting habit can usually be restored by plating macerated mycelium thickly in a thin layer of agar in capped bottles where evaporation is prevented. Potato agar with 1 percent each of lactose and dextrose, string bean agar, and malt agar are especially suitable for fruiting.

During the early part of the study of this fungus, isolates were obtained from the several host plants from as many localities as possible in the hope that on a uniform substratum the spores would show less variation in size than on the host plants and that possible host races of the fungus might be distinguished. This hope was completely disappointed. The mean length of a random sample of spores from one culture has been found as low as 13μ and from another as high as 18μ. While spores from some isolates appear to differ persistently from those of other isolates, these differences do not appear to be related to the host sources.

The morphology of the pycnidia of the *Stagonospora* stage in cultures shows remarkable diversity. This was especially true in one collection of 26 isolates from *Stagonospora* spores. When these isolates had been brought into fruiting habit and spores were plated in thin layers of agar, pycnidia were produced with little mycelium, with white mycelium, or in a black stromatic growth. In occasional cultures the pycnidial wall was very thick, like that mentioned and figured by Volkart (*46, fig. 4*) on *Trifolium repens*. In some cultures pycnidia had no ostioles or beaks; in others beaks were equal in length to the diameter of the pycnidium and opened at the surface of the agar or were oriented in all directions. Light was not found to stimulate the development of pycnidia or to influence their orientation.

The *Phoma* fruiting form of the fungus was not recognized in culture until after a collection of isolates from the *Phoma* stage and the ascigerous stage of the fungus had been obtained. It was first found in a group of cultures that had remained for some time by a north window in winter. Since it seemed likely that the low temperature had determined this development, poured plates were made from *Phoma* spores from three of the isolates and placed in incubators maintained at temperatures through the range within which the fungus had fruited freely in previous trials. After 2 weeks the plates held at 8° C. had the *Phoma* stage only; plates at 12° had only the *Phoma* stage in plates of one isolate, but both stages in plates of the other two isolates; at 16° the isolate having only the *Phoma* stage at the lower temperature now had both stages, while the other two isolates had only the *Stagonospora* stage; at 20° and 24° all cultures had only the *Stagonospora* stage. After some of the cultures had been removed from the 8° incubator and left at room temperature for 4 days, some of the pycnidia were found to have a distinct mass of *Stagonospora* spores at the bottom of the spore mass in the pycnidia, showing that with the change of temperature the pycnidia can respond with a change in the character of the spores produced. Not all pycnidia responded in this manner, nor were the two kinds of spores mixed.

Following this experiment, plates were poured from *Stagonospora* spores from old isolates—one from the fungus on sweetclover, three from white clover, and one from alsike clover—and these were placed in the same incubators. The isolate from sweetclover produced pycnidia with both spore forms at 8° C., and one of the isolates from *Trifolium repens* produced both forms at both 8° and 12°. Another isolate from white clover at 12° produced in some small pycnidia the first intergrading series of spores that has been found containing all intermediates possible between the two spore forms. The other isolates produced only the *Stagonospora* form at all temperatures. This limited experimental work serves only to indicate that low temperature is required for the development of the *Phoma* form, though all isolates did not respond alike. The presence of the two spore forms in the same pycnidium, as produced in these cultures, has not been found upon the host plant.

One point of difference in the method of discharge of the two kinds of spores from pycnidia deserves mention. The *Stagonospora* spores are discharged in threads or in slimy masses when water is present, while the *Phoma* conidia have not been seen to exude in masses in water but scatter singly. This difference in behavior seems to be associated with a characteristic difference in the development of the

contents of the pycnidium. The formation of the pycnidial cavity in the *Stagonospora* form is not precisely like that described by Dodge (*17*) in certain fungus parasites of fruits. The inception of the cavity is at or near the wall beneath the ostiole rather than at the center. This region is marked by the development of a small mass of material that stains brilliantly by the Gram method. Whether this material is the product of the disintegration of cells or is produced by cells is not clear. In culture several of these brightly staining spots may appear in the same pycnidium, and as many ostioles develop. Even on the host one pycnidium has been found having an ostiole on each surface. While there is no evidence to indicate that this staining material swells and presses against the pycnidial wall prior to spore discharge, it does form at the ostiole a plug which must absorb water from the exterior, and thus it may form the slime in which the extruded spores are at first embedded. On the other hand, this staining material has not been found in pycnidia of the *Phoma* form, and the spore mass in the pycnidium does not appear to swell appreciably when water is applied. Spores may be extruded by growth, especially in culture, but these spore masses do not appear to be at all coherent when water is applied.

TAXONOMY OF THE FUNGUS

SYNONYMY OF THE STAGONOSPORA FORM

The first and most widely known fruiting form of the fungus discussed here has been described and named repeatedly in consequence of its wide host range and variable spore length and septation. Other fungus species belonging in the genera to which this species has been assigned have been confused with it. Ascomycetous fungi found on occasion growing in association with it have been proposed as its overwintering form. Names thus applied have been brought together in ever-lengthening synonymies by mycologists, usually without recourse to cultures and often without examination of authentic specimens of all the species included. Thus the synonymies of this fungus include a group of fungi the relationship of which needs reexamination and restatement. The work reported here, together with some examination of the cultural behavior and the morphology of some of the fungi thus confused, appears to furnish the basis, not only for a new statement of the synonymy of this fungus, but also for the disposition of some of the names that have been incorrectly included.

Among the previously proposed synonymies, five have been selected which include all of the names proposed in all of them, except names derived by transfer to the unaccepted genera *Marssonina* and *Stagonosporopsis*—transfers that add nothing of interest and will not be mentioned further. The names in these synonymies, together with two others added by the authors, are grouped in table 3 opposite the names of the host plants (column 1) under which they were first described, or of the first-mentioned host plant in the list given in the original description. The citation number and date of each description follow each binomial. In the last column of table 3 the synonyms retained or proposed by the authors are designated. A brief statement of reasons for the inclusion or exclusion of each name given in a new description follows. To facilitate reference from this discussion to table 3 and for cross-reference within the table, original names applied with or without descriptions are numbered serially, while new combinations are given the numbers assigned the original names and

such repeated numbers are enclosed in brackets. Thus the names with bracketed numbers require no discussion. The complete synonymy is designated only in the last column of table 3.

TABLE 3.—*Summary of proposed synonymies of Stagonospora meliloti*

Host[1]	No.[2]	Name of fungus, citation No., and date	Synonymy[3] proposed by—					
			Davis (*14*)	Von Höhnel (*21*)	Horsfall (*24*)	Petrak (*32*)	Săvulescu and Sandu-ville (*41, p. 204*)	Jones and Weimer
Medicago arabica Huds. (=*M. maculata* Willd.).	1	*Ascochyta affinis* Jaap (*25, p. 35*), 1916	----	----	----	----	----	S
M. lupulina L.	2	*A. medicaginis* Bres. (*6, p. 326*), 1900	----	S	----	----	----	S
	3	*Marssonia medicaginis* Voss (*47, v. 4, p. 259*), 1892.	----	----	----	S	----	S
	[9]	*Septogloeum medicaginis* (Rob. and Desm.) Ell. and Ev.,[4] 1893.	----	----	----	----	----	S
	[3]	*Stagonospora medicaginis* (Voss) Bub. (*9*), 1916.	----	----	----	S	----	S
M. sativa L.	4	*Diplodina medicaginis* Oud. (*30, p. 884*), 1903	----	----	----	S	----	----
	5	*D. medicaginis* Oud. var. *phyllobia* Bub. (*9*), 1916.	----	----	----	S	----	S
	6	*Gloeosporium medicaginis* Ell. and Kell. (*20, p. 104*), 1887.	----	S	----	----	----	S
	7	*Pseudoplea briosiana* (Poll.) Höhn. (*22*), 1918	----	----	----	S	----	----
	8	*Septoria allantoidea* Berk. and Curt. (*4*), 1874	----	S	----	----	----	----
	[8]	*Rhabdospora allantoidea* (Berk. and Curt.) Sacc. (*38, v. 3, p. 586*), 1884.	----	----	----	----	----	----
	9	*Septoria medicaginis* Rob. and Desm. (*15*), 1847.	----	S	----	S	S	S
	[9]	*Stagonospora medicaginis* (Rob. and Desm.) Höhn. (*21*), 1910.	----	N	----	----	S	S
Melilotus alba Desr.	10	*Ascochyta caulicola* Laub. (*27*), 1903	----	----	----	----	S	----
	11	*A. lethalis* Ell. and Barth.,[5] 1903	----	S	----	S	----	----
	[12]	*A. meliloti* (Trel.) Davis (*13*), 1919	----	S	----	S	----	----
	12	*Gloeosporium meliloti* Trel. (*44*), 1883	----	S	----	S	----	----
	[12]	*Marssonia meliloti* (Trel.) Sacc. (*38, v. 3, p. 77*), 1884.	----	----	----	S	----	----
	[13]	*Septoria meliloti* (Lasch) Sacc. (*39*), 1889	----	----	----	S	S	S
	13	*Sphaeria meliloti* Lasch,[6] 1842	----	----	----	S	S	S
	14	*Stagonospora carpathica* Bäumler (*3*), 1888	----	----	----	S	S	----
Trifolium alpestre L.	[13]	*S. meliloti* (Lasch) Petr. (*32*), 1919	----	----	N	N	N	N
	15	*Septoria compta* Sacc. (*37, fig. 89*), 1877	S	----	----	S	S	S
	[15]	*Stagonospora compta* (Sacc.) Died. (*16*), 1912	S	----	----	----	S	S
T. pratense L.	16	*Ascochyta confusa* Bub. (*8*), 1905	S	----	----	----	----	NN
	17	*A. trifolii* Bond. and Trus. (*45*), 1913	S	----	S	----	----	----
	18	*Gloeosporium trifolii* Pk. (*31*), 1880	S	----	----	----	----	----
	19	*Phleospora trifolii* Cav. var. *recedens* C. Massalongo (*29*), 1889.	S	----	----	----	----	----
	[19]	*Ascochyta trifolii* Siem. (*42*), 1914	S	----	S	----	----	----
	20	*Pseudoplea trifolii* (Rostr.) Petr. (*32, v. 19, pp. 28–29*), 1921.	----	----	----	S	----	----
	[21]	*Rhabdospora trifolii* (Ell.) Sacc. (*38, v. 3, p. 586*), 1884.	----	----	----	----	----	----
	21	*Septoria trifolii* Ell. (*18*), 1882	----	----	S	S	----	----
T. repens L.	22	*Ascochyta volkartii* Bub. (*28, p. 190*), 1910	----	----	----	----	----	NN
	23	*Phleospora trifolii* Cav. (*11*), 1889	S	----	----	S	S	S
	[23]	*Septoria trifolii* Cav. (*10*), 1888	----	----	S	----	----	S
	24	*Stagonospora dearnessii* Sacc. (*38, v. 10, p. 333*), 1892.	S	----	----	S	S	S
	25	*S. trifolii* Fautr. (*36*), 1890	S	----	----	S	S	S
	26	*S. trifolii* Ell. and Ev. (*19, p. 82*), 189	----	----	----	S	S	S

[1] Host plant first mentioned in original description.

[2] Serial numbers indicate original names proposed or given with descriptions; numbers in brackets indicate new combinations.

[3] N designates name chosen; S, synonym; NN, nomen nudum. The complete synonymy accepted by the authors is designated in the last column.

[4] ELLIS, J. B., and EVERHART, B. M. NORTH AMERICAN FUNGI [EXSICCATI]. Ser. 2, No. 2959. *Septogloeum medicaginis* (R & D) E & E. 1893.

[5] ELLIS, J. B., and EVERHART, B. M. FUNGI COLUMBIANI [EXSICCATI]. No. 1808. *Ascochyta lethalis* Ell. and Bart. 1903.

[6] KLOTZSCH, J. F. HERBARUM VIVUM MYCOLOGICUM [etc., EXSICCATI]. No. 370. *Sphaeria meliloti* Lasch. 1842.

NOTES ON SYNONYMS OF STAGONOSPORA MELILOTI (LASCH) PETR.

Since it appears that Petrak (*32*), in 1919, assigned the earliest specific name in the proper genus to the fungus considered here, his combination, *Stagonospora meliloti*, is the name that should be retained. Although type material has not been found for examination, no good reason for superseding the well-established name appears.

The following notes refer to synonyms of *Stagonospora meliloti* as given in table 3.

(1)[4] *Ascochyta affinis* Jaap.—In writing this description, Jaap appears not to have been aware that the fungus had previously been distributed by Vestergren [5] under the name *Marssonina medicaginis* (Voss) Magnus. Moreover he notes at the end of his description that *Septoria medicaginis* Rob. and Desm. may be the same fungus, which is indeed the case.

(2) *Ascochyta medicaginis* Bres.—Included from description and from examination of Dearness' collection.[6]

(3) *Marssonia medicaginis* Voss.—Bubák (*9*), who examined the original collection by Voss appears to show beyond doubt that this name belongs in the synonymy.

(5) *Diplodina medicaginis* Oud. var. *phyllobia* Bub.—The material from which this variety was described is stated to have been contributed by J. Lind for the "Fungi Imperfecti Exsiccati." However, it is not found in that collection either under the name assigned by Bubák or that given by Lind. Thus this fungus has not been available for examination, nor has the new varietal name been found cited elsewhere. The varietal description does not appear to define any character distinguishing the fungus either from the species to which it is subordinated or from that under consideration here. Therefore, the useless name is placed in this synonymy.

(6) *Gloeosporium medicaginis* Ell. and Kell.—Included after examination of the type and of a collection by Kellerman.[7]

(9) *Septoria medicaginis* Rob. and Desm.—Included after examination of the type.[8]

(15) *Septoria compta* Sacc.—Illustration and description leave no reasonable doubt of its identity.

(23) *Phleospora trifolii* Cav.—Included after examination of the type, kindly furnished to the writers by Dr. G. Pollacci.

(24) *Stagonospora dearnessii* Sacc.—The name given by Saccardo to *Stagonospora trifolii* Ell. and Ev. because the species name *trifolii* had been used previously in that genus. The description leaves no reasonable doubt of the identity of the fungus.

(25) *Stagonospora trifolii* Fautr.—The mention of the papillate pycnidia is good evidence of its identity, even if Allescher (*2*) had not mentioned its close resemblance to the two preceding species. No material has been found for examination.

(26) *Stagonospora trifolii* Ell. and Ev.—See (24) above.

[4] Numbers not italicized, used before fungus names, are the numbers used before these names in table 3.

[5] VESTERGREN, T. MICROMYCETES RARIORES SELECTI [EXSICCATI]. No. 1541. *Marssonina medicaginis* (Voss) Magnus. 1906.

[6] KABÁT, J. E., and BUBÁK, F. FUNGI IMPERFECTI [EXSICCATI]. No. 667. *Ascochyta medicaginis* Bres. 1911.

[7] ELLIS, J. B., and EVERHART, B. M. NORTH AMERICAN FUNGI [EXSICCATI]. Ser. 2, No. 2270. *Gloeosporium medicaginis* Ell. and Kell. 1889.

[8] DESMAZIÈRES, J. B. H. J. PLANTES CRYPTOGAMES DE FRANCE [EXSICCATI]. No. 1728. *Septoria medicaginis* Rob. 1848.

NOTES ON NAMES INCORRECTLY INCLUDED IN SYNONYMIES OF STAGONOSPORA MELILOTI

The disposal of names (table 3) that are not included properly in the synonymy is as follows:

(4) *Diplodina medicaginis* Oud.—Undoubtedly a synonym of *Ascochyta imperfecta* Pk. (*43, p. 712, footnote*).

(7) *Pseudoplea briosiana* (Poll.) Höhn.—See (20) below.

(8) *Septoria allantoidea* Berk. and Curt.—Could not be certainly determined by Brooks (*43, p. 713, footnote*) as identical with *Ascochyta imperfecta* Pk. upon examination of the type material, though the meager original description admits this possibility. A fungus identified as this has been distributed under the name *Rhabdospora allantoidea* (B. and C.) Sacc.[9] This fungus has spores up to 20μ in length and can hardly be *A. imperfecta*, nor has it characters which identify it as the fungus under consideration here.

(10) *Ascochyta caulicola* Laub.—In the course of the examination of *Ascochyta* on sweetclover, two species have been distinguished. First, a ubiquitous species that produces in nature and in culture an ascigerous stage identified as *Mycosphaerella lethalis* Stone. This is *Ascochyta lethalis* Ell. and Barth. The second species is less abundant and is not distinguished from the first by spore measurement or pycnidial character on the host plant. It is distinguished by cultural characters, by the hypertrophy of some of the host plants like that described by Laubert (*27*) as caused by *A. caulicola*, and by the absence of an ascigerous stage in culture and on the host so far as it has been observed at present. Under these circumstances, pending further investigation, *Ascochyta caulicola* Laub. is considered to be a valid name applying to the fungus that causes hypertrophy of the host plant, and not a synonym of *A. lethalis*.

(11) *Ascochyta lethalis* Ell. and Barth.—The imperfect stage of *Mycosphaerella lethalis* Stone, not often found fruiting abundantly on the living host. The ascigerous stage of this fungus has been obtained repeatedly in cultures on sweetclover stems stored at low temperatures. This fungus fruits rarely on leaves, which, when abundantly infected, soon shrivel and fall, thus behaving differently from leaves infected with *Stagonospora meliloti*. However, an excellent fruiting collection was made in the autumn on leaves of *Melilotus officinalis*, and another in the spring on *M. dentata*. The pycnidia on these leaves, like those on the stems, had no beaks.

(12) *Gloeosporium meliloti* Trel., or *Ascochyta meliloti* (Trel.) Davis.—Appears to have been placed correctly by Davis (*14*) as a synonym of *A. lethalis*, after examination of the original collection.

It is noted here that the fungus collected and distributed by Carlton under the synonym *Marssonia meliloti* Trel. is not that fungus, but is *Stagonospora meliloti*.

(14) *Stagonospora carpathica* Bäumler.—This name has been applied several times to the *Stagonospora* collected on *Melilotus* in the United States, in spite of the fact that conidiophores 8μ to 10μ long and 2μ in diameter are specified in the original description. No conidiophores have been seen in any specimen of *Stagonospora* examined.

[9] ELLIS, J. B., and EVERHART, B. M. FUNGI COLUMBIANI [EXSICCATI]. No. 1060. *Rhabdospora allantoidea* (B. and C.) Sacc. 1897.
——— and EVERHART, B. M. NORTH AMERICAN FUNGI [EXSICCATI]. Ser. 2, No. 3462. *Rhabdospora allantoidea* (B. and C.) Sacc. 1896.

Thus the name must be excluded from the synonymy. The writers have not seen any fungus on this plant to which the description applies.

(16) *Ascochyta confusa* Bub.—This name, suggested as a possible synonym, has been found without published description. The original collection has not been found. Therefore the binomial must be discarded as a nomen nudum.

(17) *Ascochyta trifolii* Bond. and Trus.—See *S. recedens* below.

(18) *Gloeosporium trifolii* Pk. See section on *Stagonospora recedens*, this page.

(19) *Phleospora trifolii* Cav. var. *recedens* C. Massalongo. See section on *Stagonospora recedens*, this page.

(20) *Pseudoplea trifolii* (Rostr.) Petr.—Too well known from recent studies to leave any room for suspicion that it has an imperfect fruiting stage.

(21) *Septoria trifolii* Ell.—Readily excluded from consideration by the size and shape of the spores as given in the description. Found upon dead leaves, it is probably a saprophyte.

(22) *Ascochyta volkartii* Bub.—This name, suggested as a possible synonym, has been found without published description. The original collection has not been found. Therefore the binomial must be discarded as a nomen nudum.

Stagonospora recedens (C. Massalongo) n. comb.

Three species (table 3, Nos. 17, 18, and 19) named as occurring on *Trifolium pratense* have been examined critically. The type collection of the oldest of these, *Gloeosporium trifolii* Pk., lent to the writers by the New York State Museum, has been found sterile. However, a fungus under this name corresponding well to the description and collected by Dearness [10] and several collections by J. J. Davis are deposited in the herbarium of the University of Wisconsin. These have been examined. Type material of *Ascochyta trifolii* Bond. and Trus. has been contributed by Dr. Bondartzeff. *Phleospora trifolii* Cav. var. *recedens* C. Massalongo has been seen in a collection by Krieger.[11] These collections appear to be of the same fungus, which is distinguished sharply from *Stagonospora meliloti* by the absence of the characteristic beak (fig. 3, *B*). Some of the pycnidia have short isodiametric papillae, as described by the authors, but they are not typical of those found in *S. meliloti*. In addition to this fungus, the collections by Davis and by Bondartzeff have the fungus that appears to have been incorrectly included with *Sporonema phacidioides* Desm. by Saccardo (*38, v. 25, p. 528*). Thus *S. meliloti* appears not to occur on red clover. The closely similar fungus on this host to which the three names given above have been applied appears to belong in the genus *Stagonospora*, especially in view of Korkhyrakoff's report (*26*) of spores with more than one septum. The species name applied by two authors to the fungus is not available, since it has been used for another species in that genus. Therefore, the varietal name applied by Massalongo is chosen for the species name of this fungus. Thus the name suggested is *Stagonospora recedens* (C. Massalongo) n. comb.

[10] Bartholomew, E. Fungi Columbiani [exsiccati]. Cent. 46, No. 4538. *Gloeosporium trifolii* Pk. 1915.

[11] Krieger, W. Fungi Saxonici [exsiccati]. No. 1072. *Phleospora trifolii* Cav. var. *recedens* C. Mass. 1895.

The description by Trusova (*45*) appears to be the most adequate when amended by the mention of the occurrence of spores with two or more septa.

TAXONOMY OF THE PHOMA FORM

The fruiting structure produced on stems of sweetclover in the autumn after the larger spored structures have ceased to develop belongs beyond doubt in the genus *Phoma*. Spores of this *Phoma* stage germinate in culture media and produce the *Stagonospora* stage in culture.

Two species in the genus *Phoma* have been described on sweetclover. One of these, *Phoma oleracea* Sacc. (*40, p. 549*), is certainly not the species at hand. Not only are the spore measurements given for the species too large, but in the type material lent to the writers by Dr. Dearness the pycnidia are found to be superficial, in striking contrast with the deeply situated pycnidia illustrated here (fig. 3, *C*.).

The description of the other species of *Phoma* on sweetclover—*Phoma meliloti* Allescher (*1*)—applies more closely to the material at hand. The minimum spore width of 0.5μ given in the description is too small for even the shrunken specimens in dry material, though these are very narrow indeed, and the pycnidia seem rather widely scattered on stems rather than gregarious as stated. The type collection of this fungus has not been seen; but a fungus distributed under this name by Krieger [12] has been examined. The collection was made in October, the right season of the year for the development of this form, and on small branches. A comparison of this material with a collection made near Madison on similar branches failed to show any differences whereby the pycnidia from the two collections could be distinguished. There were more pycnidia with characteristic flaring beaks on the Madison collection than on Krieger's collection, but a few were found on the latter. In both collections immature fruiting structures that may have been perithecia were found. Thus, on the basis of this comparison it appears that the name *Phoma meliloti* Allescher is correct as applied to the autumn fruiting structure of *Stagonospora meliloti*.

SYNONYMY OF THE ASCIGEROUS FORM, LEPTOSPHAERIA PRATENSIS SACC. AND BRIARD

The perfect stage of *Stagonospora meliloti* is well represented in European collections. It first appears in Rabenhorst,[13] under the name *Leptosphaeria dumetorum* Niessl, on *Melilotus alba*. The spores of the fungus in this collection, as measured by the authors, are 22μ to 26μ long, while a length of 18μ to 20μ is given in the description of the species. Rehm (*34*) distinguishes this collection as *Leptosphaeria dumetorum* Niessl f. *meliloti* Rehm, but gives the spore length as stated in the original species—presumably an oversight, though it is possible that Rabenhorst's collection was mixed, containing another of the several species of *Leptosphaeria* occurring on this host. Later, Berlese (*5, v. 1, pp. 55–56*) included Rehm's form in a new variety of another species, *L. eustoma* (Fries) Sacc. f. *major* Berlese, still giving the short spore measurement. While Berlese's figure (*5, table XLI,*

[12] KRIEGER, W. FUNGI SAXONICI [EXSICCATI]. No. 2284. *Phoma meliloti* Allescher. 1914.
[13] RABENHORST, L. FUNGI EUROPAEI [EXSICCATI]. No. 2238. *Leptosphaeria dumetorum* Niessl. 1877.

fig. 5) shows short, rather thick spores consistent with his measurement, still one of his three type collections,[14] in the packet that the authors have examined, contains typical material with spores more than 20μ in length. Thus this variety appears to be in part the perfect stage of *Stagonospora meliloti*.

It appears likely that the incorrect spore measurement stated by both Rehm and Berlese left the way open for the later descriptions of this species. The first correct designation of this fungus as a species appears to be *Leptosphaeria pratensis* Sacc. and Briard (*7*) described on *Medicago sativa*. The spore length is given as 28μ to 32μ. Berlese, who drew the fungus from the original collection (*5, v. 1, table LV, fig. 1*) gives the spore length as 28μ to 30μ. The authors have not seen this original material. Spores from the first collection made on alfalfa, discharged upon agar plates measured 25μ to 32μ in length, and thus were of the length stated in the original description. Spores from a collection made in the following year measured 21μ to 27μ in length, with a mean length of 23.6μ. From these data the opinion has been reached that the greater spore size stated for *Leptosphaeria pratensis* in the original description, while probably correct, is an extreme size in the species and is not a character distinguishing it from the *Leptosphaeria* previously discussed on *Melilotus alba*.

The fungus was later named *Leptosphaeria meliloti* by Hollós (*23*), who recognized (in a letter to the authors) the previous varietal designations stated above.

The collections of the fungus that have been verified by examination all being on *Melilotus* spp. except the American collection, which is stated to be on an undetermined shrubby plant, are listed with the proposed synonymy as follows:

Name:
Leptosphaeria pratensis Sacc. and Briard.
Synonyms:
L. dumetorum Niessl (in part in herbaria).
Rabenhorst, Fungi Europaei No. 2238, type.
Krieger, Fungi Saxonici No. 911.
De Thümen, Mycotheca Universalis No. 2248.
Petrak, Flora Bohemiae et Moraviae Exs. No. 2044.
Sydow, Flora Marchica (not numbered).
Vestergren, Micromycetes Rariores Selecti No. 726.
Ellis and Everhart, North American Fungi, 2d ser., No. 2614 (a).
L. dumetorum Niessl f. *meliloti* Rehm.
Sydow, Mycotheca Germanica No. 2528.
L. eustoma (Fries) Sacc. f. *major* Berlese (in part, as represented by Mycotheca Universalis No. 2248).
L. meliloti Hollós.

SUMMARY

A root rot of alfalfa, found first by the authors in California and in Wisconsin, is caused by a fungus previously known chiefly as a leaf-spotting fungus but having three fruiting forms, all of which have been described previously as independent species. This fungus is capable of producing a root rot of sweetclover also, though such root rot has been recognized but once in the field. The root rot progresses slowly, and is favored by high soil temperature. It is not readily distinguished macroscopically, but microscopically it is readily identified by staining

[14] DE THÜMEN, F. MYCOTHECA UNIVERSALIS [EXSICCATI]. No. 2248. *Leptosphaeria dumetorum* Niessl. 1884.

the fungus mycelium in roots or large stems with the Gram stain. Mycelium in leaves or in culture media is not thus stained. While the root rot develops best at a high soil temperature, the leaf spot develops abundantly at low temperature in early spring and late fall.

The chief imperfect stage of the fungus causing this root rot is defined here as a morphological species in the genus *Stagonospora*, identifiable on all of its host plants in three genera of legumes by the characteristically shaped rostrum, or beak, of the pycnidium. Cultures have been made of the fungus from nearly all of the host species known. While the fungus is variable in culture, none of the variations appear to be associated with the host origin. Some of the cultures from the more important host sources will infect sweetclover roots, though in varying degree. Cultures from sweetclover vary greatly in pathogenicity when inoculated into roots of that plant.

The first and most conspicuous fruiting form of the fungus appears to be properly designated *Stagonospora meliloti* (Lasch) Petr. This fungus has a large number of synonyms, which are discussed. The second form, found thus far only on *Melilotus alba*, in the autumn, has been previously described as *Phoma meliloti* Allescher. The ascigerous form, found thus far on alfalfa and sweetclover stems, in the spring, is *Leptosphaeria pratensis* Sacc. and Briard.

For a species of *Stagonospora* on *Trifolium pratense* closely resembling this species, the new combination *Stagonospora recedens* (C. Massalongo) is suggested.

LITERATURE CITED

(1) Allescher, Andreas.
1892. verzeichnis in süd-bayern beobachteter pilze. ein beitrag zur kenntnis der bayer. pilzflora. Ber. Bot. Ver. Landshut (Bayern) 12 (Abhandl. I): 19.

(2) ———
1901. stagonospora dearnessii. *In* Rabenhorst, L., Kryptogamen-Flora von Deutschland, Österreich und der Schweiz, Aufl. 2. Bd. 1, Abt. 6, pp. 989–990. Leipzig.

(3) Bäumler, J. A.
1888. fungi schemnitzenses. ein beitrag zur ungarischen pilzflora. Verhandel. Zool.-Bot. Gesell. Wien 38: 707–718.

(4) Berkeley, M. J.
1874. notices of north american fungi. Grevillea 3: 1–17.

(5) Berlese, A. N.
1894. icones fungorum omnium hucusque cognitorum ad usum sylloges saccardianae adcommodatae. 2 v. Florentinae and Patavii.

(6) Bresadola, J.
1900. fungi aliquot saxonici novi lecti a cl. w. krieger. Hedwigia 39: 325–328.

(7) Briard, [H.]
1885. champignons nouveaux de l'aube, fasc. l. Rev. Mycol. 7: 208–212.

(8) Bubák, Fr.
1905. bericht über die tätigkeit der station für pflanzenkrankheiten und pflanzenschutz an der königl. landwirtschaftlichen akademie in taber (böhmen) in jahre 1904. Ztschr. Landw. Versuchs. Österr. 8: 513–516.

(9) ———
1916. pilze von verschiedenen standorten. Ann. Mycol. 14: 341–352, illus.

(10) CAVARA, FRIDIANO.
1888. APPUNTI DI PATOLOGIA VEGETALE (ALCUNI FUNGHI PARASITI DI PIANTE COLTIVATE). Atti Ist Bot. R. Univ. Pavia 1: 425-436, illus.
(11) ———
1889. MATÉRIAUX DE MYCOLOGIE LOMBARDE. Rev. Mycol. 11: [173]-193, illus.
(12) CREAGER, DON B.
1933. LEAF SCORCH OF NARCISSUS. Phytopathology 23: 770-786, illus.
(13) DAVIS, J. J.
1919. NORTH AMERICAN ASCOCHYTAE. A DESCRIPTIVE LIST OF SPECIES. Wis. Acad. Sci., Arts, and Letters, Trans. 19: 655-670.
(14) ———
1919. NOTES ON PARASITIC FUNGI IN WISCONSIN IV. Wis. Acad. Sci., Arts, and Letters, Trans. 19: 671-689.
(15) DESMAZIÈRES, J. B. H. J.
1847. QUATORZIÈME NOTICE SUR LES PLANTES CRYPTOGAMES RÉCEMMENT DÉCOUVERTES EN FRANCE. Ann. Soc. Nat., Bot. (3) 8: 9-37.
(16) DIEDICKE, H.
1912. DIE GATTUNG SEPTORIA. Ann. Mycol. 10: [478]-487.
(17) DODGE, B. O.
1923. ORIGIN OF THE CENTRAL AND OSTIOLAR CAVITIES IN PYCNIDIA OF CERTAIN FUNGUS PARASITES OF FRUITS. Jour. Agr. Research 23: 743-760, illus.
(18) ELLIS, J. B.
1882. NEW SPECIES OF NORTH AMERICAN FUNGI. Bull. Torrey Bot. Club 9: 73-74.
(19) ELLIS, J. B., and EVERHART, B. M.
1892. NEW SPECIES OF FUNGI FROM VARIOUS LOCALITIES. Acad. Nat. Sci. Phila. Proc. 1891: 76-93.
(20) ——— and KELLERMAN, W. A.
1887. NEW KANSAS FUNGI. Jour. Mycol. 3: 102-105.
(21) HÖHNEL, FRANZ V.
1910. FRAGMENTE ZUR MYKOLOGIE, NR 547. SPORONEMA DESM. Sitzber. Akad. Wiss. Wien (Abt. 1) 119: 642-648.
(22) ———
1918. ÜBER PLEOSPHERULINA BRIOSIANA (POLLACCI). Ann. Mycol. 16: 162-163.
(23) HOLLÓS, LASZLO.
1926. UJ GOMBÁK SZEKSZÁRD VIDÉKÉRÖL. Math. és Term. Kozlem. Kotet 35, 64 pp., illus.
(24) HORSFALL, JAMES G.
1930. A STUDY OF MEADOW-CROP DISEASES IN NEW YORK. N. Y. (Cornell) Agr. Expt. Sta. Mem. 130, 138 pp., illus.
(25) JAAP, OTTO.
1916. BEITRÄGE ZUR KENNTNIS DER PILZE DALMATIENS. Ann. Mycol. 14: 1-44.
(26) KORKHYRAKOFF, M. K.
1932. MYCOLOGICAL NOTES. Trudy Zashch. Rast. (Bull. Plant Protection), Leningrad 1: 125-129.
(27) LAUBERT, R.
1903. ASCOCHYTA CAULICOLA. EIN NEUER KRANKHEITSERREGER DES STEINKLEES. Arb. Biol. Abt. f. Land u. Forstw. 3: 441-443, illus.
(28) MALKOFF, KONSTANTIN.
1910. ZWEITER BEITRAG ZUR KENNTNIS DER PILZFLORA BULGARIENS. Ann. Mycol. 8: 187-191.
(29) MASSALONGO, C. B.
1889. CONTRIBUZIONE ALLA MICOLOGIE VERONESE. Mem. Accad. Agr. Arti, e Com., Verona (3) 65: [133]-283, illus.
(30) OUDEMANS, C. A. J. A.
1903. CONTRIBUTIONS A LA FLORE MYCOLOGIQUE DES PAYS-BAS. Nederland. Kruidk. Arch. (3) 2: [851]-928, illus.
(31) PECK, CHARLES H.
1880. PLANTS NOT BEFORE REPORTED. N. Y. State Mus. Nat. Hist. Ann. Rept. 33: 17-34.

(32) PETRAK, F.
1919–23. MYKOLOGISCHE NOTIZEN. Ann. Mycol. 17: 64–66, 1919; 19: 28–29, 1921; 21: 334–335, 1923.
(33) RAWLINS, THOMAS ELSWORTH.
1933. PHYTOPATHOLOGICAL AND BOTANICAL RESEARCH METHODS. 156 pp., illus. New York and London.
(34) REHM, [H.]
1883. ASCOMYCETEN, FASC. XIV. Hedwigia 22: 52–61.
(35) RIDGWAY, ROBERT.
1912. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 pp., illus. Washington, D. C.
(36) ROUMEGUÈRE, C.
1890. FUNGI EXSICCATI PRECIPUE GALLICI, 5478. STAGONOSPORA TRIFOLII SP. N. Rev. Mycol. 12: 167.
(37) SACCARDO, P. A.
1877–86. FUNGI ITALICI AUTOGRAPHICE DELINEATI (ADDITIS NONNULLIS EXTRA-ITALICIS, ASTERISCO NOTATIS). 14 pp., illus. Patavii.
(38) ———
1882–1935. SYLLOGE FUNGORUM OMNIUM HUCUSQUE COGNITORUM. v. 1–25 and supplements. Patavii.
(39) ———
1889. NOTES MYCOLOGIQUES. Bull. Soc. Mycol. France 5: [115]–123.
(40) ———
1913. NOTAE MYCOLOGICAE. II. FUNGI CANADENSES. Ann. Mycol. 11: 549–553.
(41) SĂVULESCU, TR., and SANDU-VILLE, C.
1935. BEITRAG ZUR KENNTNIS DER MICROMYCETEN RUMÄNIENS. Hedwigia 75: 159–233.
(42) SIEMASZKO, W.
1914. ZAPISKI GRZYBOZNAWCZE Z GUBERNII WILENSKIEJ. TOWARSYSTWO NAUKOWE, WARSZAWA. Sprawozodania 7: 141–152.
(43) TOOVEY, F. W., WATERSON, J. M., and BROOKS, F. T.
1936. OBSERVATIONS ON THE BLACK-STEM DISEASE OF LUCERNE IN BRITAIN. Ann. Appl. Biol. 23: 705–717, illus.
(44) TRELEASE, WILLIAM.
1885. PRELIMINARY LIST OF WISCONSIN PARASITIC FUNGI. Wis. Acad. Sci., Arts, Letters, Trans. (1881–83) 6: 106–144.
(45) TRUSOVA, N. P.
1913. [FUNGUS DISEASES OF CULTIVATED WILD PLANTS IN THE TULSK'I GOVERNMENT OBSERVED DURING 1912.] [Translated title.] Bolesnei Rastenii 7: 211–217. [In Russian.]
(46) VOLKART, A.
1904. PFLANZENSCHUTZ. Landw. Jahrb. Schweiz 18: 94–97, illus.
(47) VOSS, WILHELM.
1892. MYCOLOGIA CARNIOLICA. EIN BEITRAG ZUR PILZKUNDE DES ALPENLANDES. VIERTERTHEIL. FUNGI INFERIORES, MYCELIA, MYXOMYCETES. v. 4, pp. [219]–302. Berlin.

HISTOLOGY OF APPLE FRUIT TISSUE IN RELATION TO CRACKING[1]

By LEIF VERNER
Horticulturist, Idaho Agricultural Experiment Station

INTRODUCTION

Extensive loss often is incurred in the production of various fruits, notably sweet cherries (*Prunus avium* L.) and certain varieties of apple (*Malus sylvestris* Mill.), as a result of cracking of the skin and fleshy tissues sometime prior to harvest. Recent investigations have shown that in some of these fruits cracking is associated with abnormal acceleration of fruit growth or swelling as a result of a marked increase in water supply to the tissues. Verner and Blodgett (*12*)[2] found that cracking in sweet cherries was induced by an osmotic absorption of water through the fruit skin when this remained wet during prolonged periods of rain; and Verner (*10*) concluded that cracking in Stayman Winesap apples is promoted by increased water supply to the fruit tissues as a result of greatly depressed transpirational water loss under conditions of high humidity. In general, there seems to be a tendency for both cherries and apples to become increasingly susceptible to cracking as the fruits approach maturity.

Among different varieties of the same kind of fruit striking differences in tendency to crack have been observed. Bing, Lambert, and Napoleon sweet cherries are highly susceptible to this injury, while Republican, Eagle, and many other varieties are more or less free of it (*9*). Stayman Winesap apples sometimes crack severely, while apples of other varieties on nearby trees do not crack at all. Even among different individual fruits on the same tree remarkable differences in tendency to crack may be observed, some fruits cracking severely rather early in the season while others are still sound at maturity.

We may infer from such observations that some varieties, in general, and certain specific fruits of those varieties in particular, are in some manner peculiarly predisposed to cracking. Evidently those forces tending to promote unusually rapid swelling of the inner tissues through increased hydration are not alone responsible for this phenomenon. Some fruits, apparently, are able to accommodate these rapid rates of enlargement by corresponding growth or stretching of the peripheral tissues, while other fruits are not so adapted. The environmental influences that tend to promote cracking must, therefore, be regarded as merely contributory.

The present study was undertaken with the hope of finding a histological basis for the explanation of these diverse tendencies of different fruits to crack. Sections of fruit tissue of the highly susceptible Stayman Winesap apple were compared with similar tissues of several varieties in which cracking is rare. The latter varieties included Winesap, Winter Banana, Grimes Golden, Gano, and

[1] Received for publication April 26, 1938; issued December 1938. Research Paper No. 168, of the Idaho Agricultural Experiment Station.
[2] Italic numbers in parentheses refer to Literature Cited, p. 823.

Arkansas. Especially careful examination was made of tissues adjacent to cracks in Stayman Winesap; and specimens of this variety that had cracked were compared with specimens which, although growing in the same orchard or on the same tree, had remained sound.

In a fruit such as the apple, which approximates a spherical shape, the most rapid growth in a tangential direction must occur in the outermost tissues. If growth or tangential stretching in these parts is unable to keep pace with growth or swelling of the underlying tissues (largely cortex in the apple), cracking may be expected to occur in the fruit periphery. The nature of tangential growth or stretching of the epidermal and hypodermal layers, roughly constituting the fruit "skin," is, therefore, of special interest in a study of cracking in this fruit. The relative positions of these tissues and the manner in which they are involved in cracking are shown by the photomicrograph in plate 1, *A*. Major consideration has been given to these tissues.

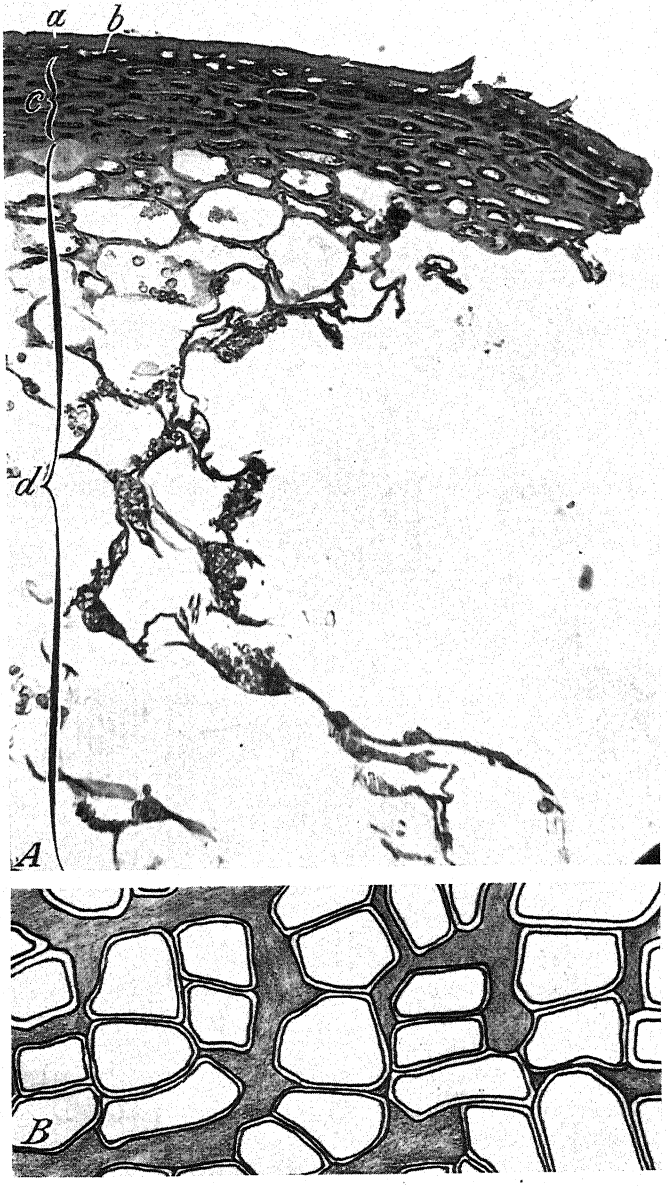
REVIEW OF LITERATURE

Since a review of literature on the subject of fruit cracking has been published recently (*10*), only such papers as have a direct bearing on the present study are considered here.

Tetley (*8*), in an anatomical study of Bramley Seedling apple, found that both cell division and cell growth continued later in the hypodermal layer of cells than in any other part of the cortex; and epidermal cells, she states, may continue to divide as long as the fruit is growing. As a rule, however, cell division in these regions decreases with advancing maturity, and much of the tangential stretching to which the peripheral tissues are subjected late in the season seems to be accommodated by a change in the direction of the long axes of cells in this region. In very early stages of development, when the apple is newly "set," the epidermal cells are somewhat brick-shaped, with their long axes lying in a radial plane; but, as growth proceeds, these cells seem gradually to yield to stretching until, at maturity, their long axes lie in a tangential plane. In the hypodermal cells also the tangential dimensions increase more radiply than the radial dimensions in the later growth stages. This change in shape of epidermal and hypodermal cells, as a result of tangential stretching, might conceivably provide for a considerable increase in surface area of the fruit without any appreciable cell growth having taken place.

Tetley states that the epidermis of Cox Orange Pippin, an English variety of apple that cracks readily, is characterized by deposition of cutin on the outer tangential, the radial, and the inner tangential walls of the epidermal cells. She considers that this type of cuticle offers less resistance to tangential stretching than a type of cuticle that covers only the outer tangential walls, or the outer tangential and radial walls of the epidermal cells. She seems to consider the type of cuticle of Cox Orange Pippin at least partly responsible for the tendency of this variety to crack.

Zschokke (*13*) observed that hairlike epidermal cells present in very young apples commonly are broken in the early growth stages of the fruit, leaving in the epidermis minute openings which subsequently become suberized and give rise to lenticels. Tetley (*8*), who also observed these hairlike structures, states that upon collapse of such turgid cells in a stretched epidermis small cracks in the skin may appear.



A, Transverse section showing one side of a shallow crack in a Stayman Winesap apple and the tissues involved in such cracking. × 125. *a*, Cuticle; *b*, epidermis; *c*, hypodermis; *d*, cortex. *B*, Tangential section through epidermis of Stayman Winesap apple showing cut-in (shaded portions) deposited between groups of epidermal cells. Traced from a photomicrograph. × 500.

According to Sorauer (*7*) cork formation usually occurs in the tissues subjacent to such small cracks, and this cork development may result in local russeting of the fruit skin. In subsequent growth of the fruit, cracks are likely to appear in such regions because of nonuniform swelling of the russet and the healthy areas.

Verner (*10*) considers that lenticel hypertrophy, which seems to be induced by the same environmental conditions that promote cracking, may sometimes constitute an initial stage in the development of cracks in Stayman Winesap apples. He points out that cracks in this variety occur most commonly in areas characterized by some abnormality of the peripheral tissues, such as russeting, lesions of apple scab (*Venturia inaequalis*), sunburn, or high skin coloration—conditions that seem to decrease the extensibility of these tissues.

Kertesz and Nebel (*3*), in a study of varietal differences in susceptibility to cracking in sweet cherries, found that those varieties that cracked most readily had smaller cells and thus, presumably, more cell-wall material than those resistant to cracking. When finely divided pulp of different varieties was placed on wet filter paper in funnels it was found that a relatively large percentage of the juice was retained by pulp of the varieties most susceptible to cracking, while in the noncracking sorts much of the juice passed through the filter. The greater retention of liquid by pulp of the varieties that crack badly was attributed to the imbibitional properties of the greater amount of colloidal substance in these fruits. These colloids of the cell walls, they conclude, probably exert a greater influence on absorption of water by the fruit in periods of rain than do the osmotically active constituents of the cell vacuoles; and they consider that varietal differences in colloid content serve to explain differences in their susceptibility to cracking.

METHODS OF PROCEDURE

The apple tissue samples used in this study were secured in 1933 and 1934 from nearly mature fruit grown at the Kearneysville Experiment Farm of the University of West Virginia. By means of a razor blade, wedge-shaped pieces of tissue were cut from the apple cheek in such a manner that each consisted of a 5-mm square of fruit skin and tapered on radial planes toward the core. In length these pieces of tissue ranged from 1 to 3 cm, the longer pieces usually traversing the cortex and including a portion of the pith of the apple.

As soon as a tissue sample was removed from an apple it was placed in a formalin-alcohol killing and fixing solution. The samples were prepared for embedding in paraffin by a routine procedure of dehydration in an alcohol series of increasing concentrations from 35 to 100 percent, and by clearing in a series of alcohol-xylol mixtures of increasing concentrations of xylol from 33⅓ to 100 percent. Paraffin with a melting point of 52° C. was used for embedding. Transverse sections 10 to 15 microns thick were cut with a rotary microtome, and these sections were stained with Delafield's haematoxylin and Sudan III according to the schedule recommended by Baker (*1*). The stained sections were mounted in glycerin jelly.

Photomicrographs were taken by means of a euscope equipped with a camera attachment. Length of exposure of the plates was controlled

through the use of a camera shutter attached to the substage of the microscope. A carbon-arc lamp served as a source of light.

Most of the sectioning, staining, and study of these tissues was carried on in 1935 and 1936 at the University of Idaho.

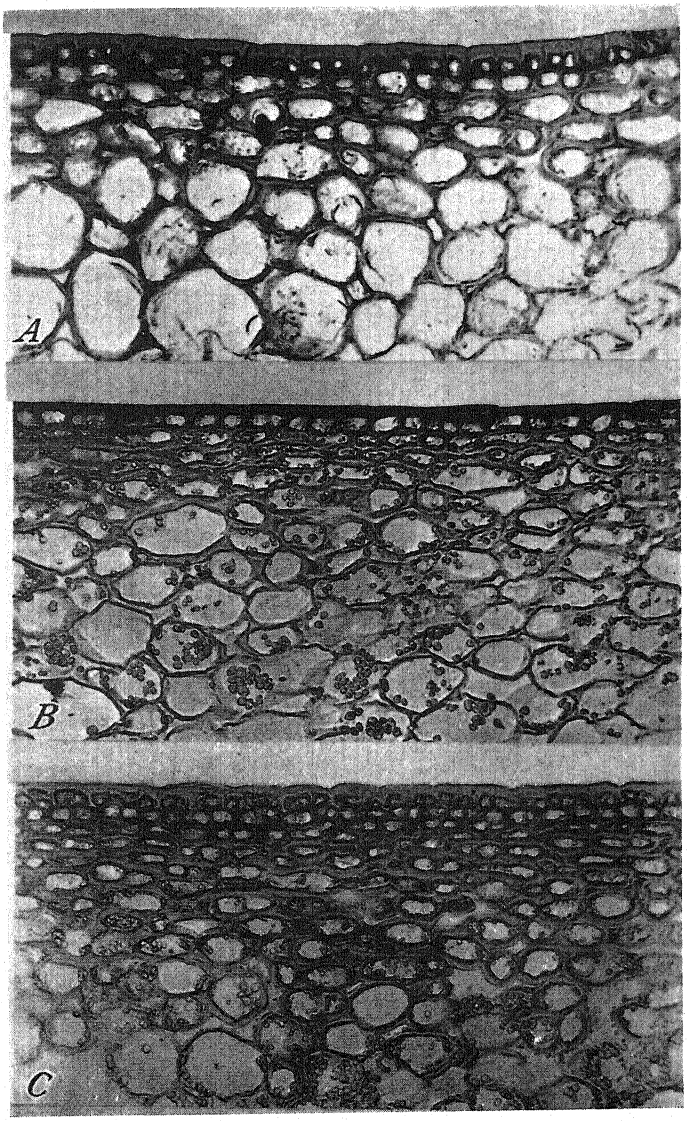
OBSERVATIONS

DIFFERENCES IN THE PERIPHERAL TISSUES OF CRACKING AND NONCRACKING APPLES

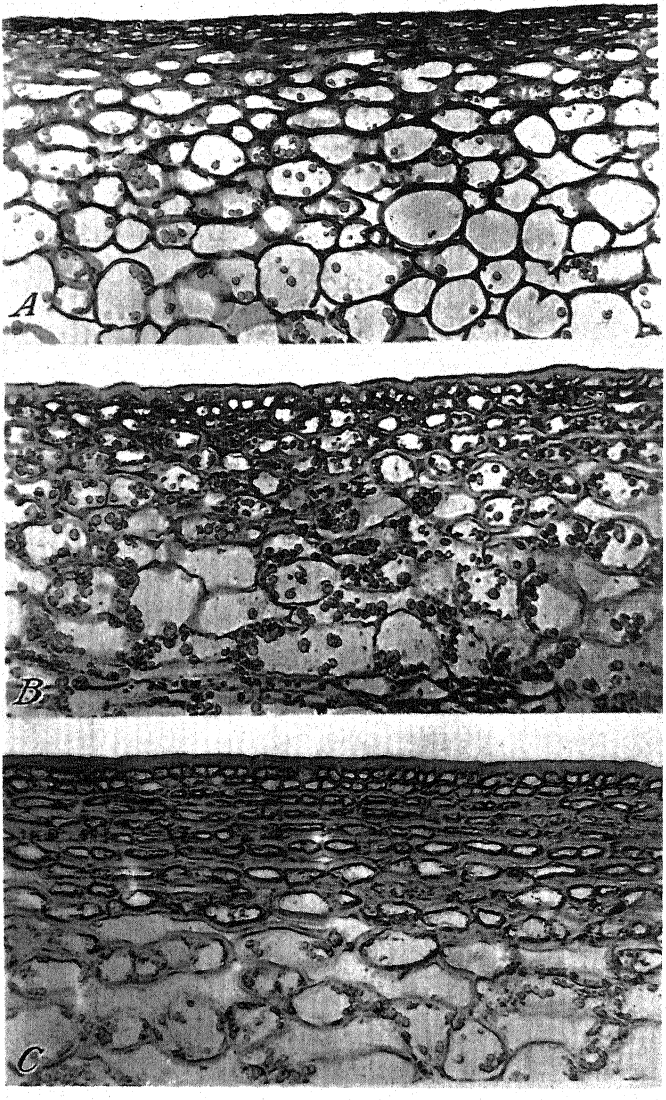
There were observed no consistent differences in structure of the cuticle or of the epidermis that might help to explain the tendency of some fruits to crack. In all varieties examined the cells of the epidermis had been pulled apart into small groups, as shown in plate 1, *B*, and the epidermis had remained intact only by virtue of increase in the cuticle, which had been projected into the interstices formed between the radial walls of cells that had been separated. In many cases cells of the epidermis were completely embedded in cutin and obviously were incapable of further growth. The development of this condition of the epidermis probably is attributable to premature cessation of division and growth in the cells of this layer, with a subsequent separation of these cells, one from another, as the epidermis is called upon to cover a larger and larger area of fruit surface. Preservation of the epidermal layer as a continuous, unbroken covering over the fruit is, therefore, dependent primarily upon continued deposition and stretching of the cutin that holds the epidermal layer intact. Since deposition of cutin may thus supplement deficient growth of the epidermis, the failure of the epidermal tissue itself to maintain an adequate growth rate cannot be regarded as contributing materially to the susceptibility of a variety to cracking. This view is supported by the fact that in varieties resistant to cracking, as well as in Stayman Winesap, the epidermis behaves in this manner.

In the hypodermal region of these apples striking differences were observed among the varieties studied, and these differences appear to be intimately related to the phenomenon of cracking. In Stayman Winesap the cells of the hypodermis, like those of the epidermis, showed unmistakable evidence of a deficient growth rate in the later stages of fruit enlargement; but this was not so of the varieties resistant to cracking. With few exceptions, the Stayman Winesap tissues examined were characterized by a more clearly defined hypodermal layer than was observed in any other of the varieties studied. In Stayman Winesap this layer consisted of much smaller and thicker-walled cells than were found in the subjacent part of the cortex, and these two regions usually were quite distinct. In most of the other varieties the hypodermal layer usually lacked a definite outline, the somewhat smaller cells of this region merging imperceptibly with the larger cortical cells. These different types of hypodermis are illustrated in plates 2 and 3, showing partial cross-sections of tissue from apparently normal fruits of Arkansas, Gano, Winesap, Grimes Golden, Winter Banana, and Stayman Winesap.

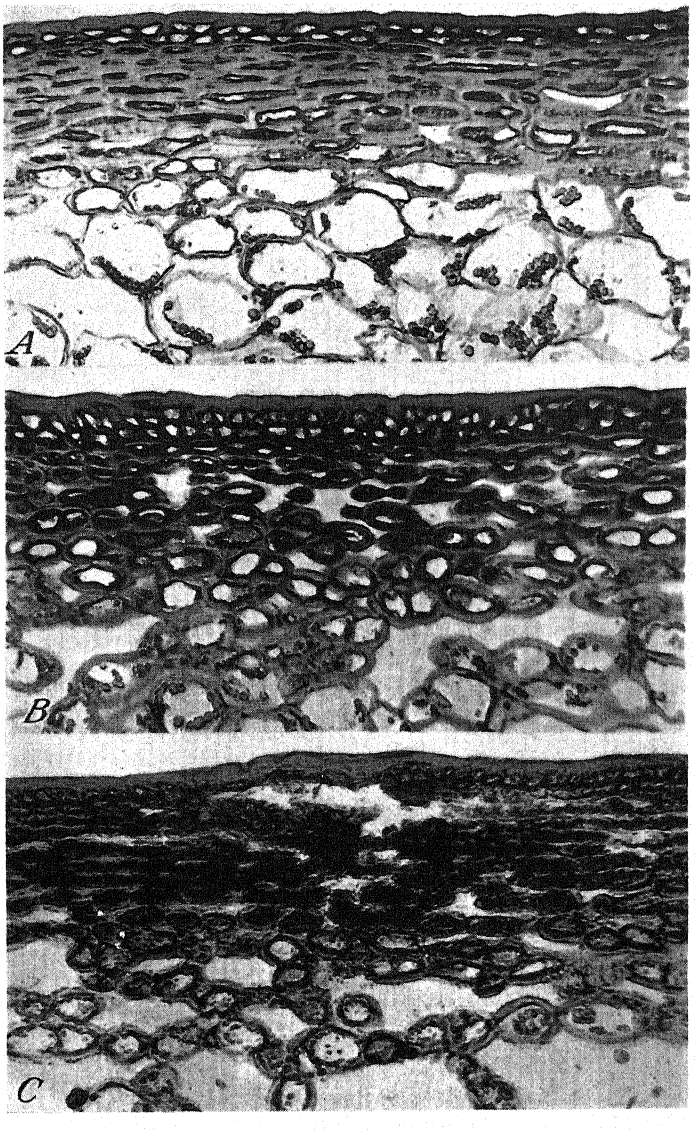
As shown in plate 3, *C*, tangential dimensions of the hypodermal cells of Stayman Winesap often are much greater than the radial dimensions, indicating that late growth of the fruit cortex was accommodated in the peripheral region principally by tangential stretching of the hypodermal cells rather than by cell growth. In the other



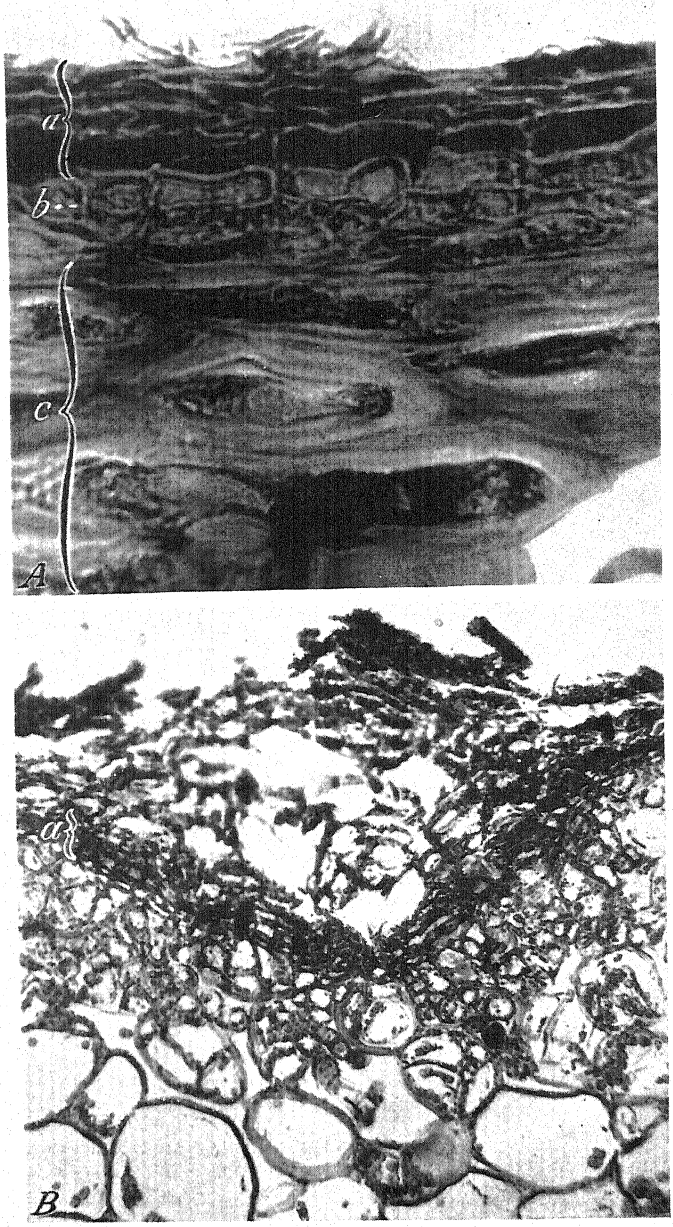
Transverse sections showing epidermal, hypodermal, and outermost cortical tissues of apple varieties resistant to cracking: *A*, Arkansas; *B*, Winter Banana; *C*, Winesap. × 125.



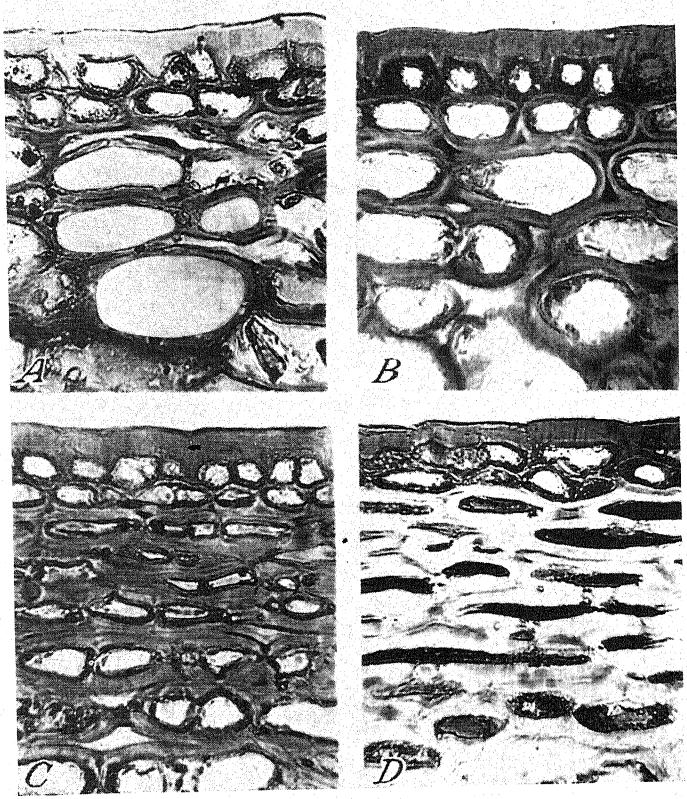
Transverse sections showing epidermal, hypodermal, and outermost cortical tissues of apple varieties: *A*, Gano (resistant to cracking); *B*, Grimes (resistant to cracking); *C*, Stayman Winesap (susceptible to cracking). × 125.



Transverse sections of Stayman Winesap apples showing modifications of normal peripheral tissues: *A*, adjacent to a crack; *B*, in a sunburned area; *C*, in an area probably injured by a lime-sulphur fungicide. × 125.



Transverse sections of Stayman Winesap apples showing: *A*, tangential elongation of hypodermal cells under cork tissue (*a*, cork; *b*, cork cambium; *c*, hypodermis). × 600. *B*, Hypodermal cells cut off from cortex by a deep layer of cork (darkly stained zone—*a*) and incipient cracking in the hypodermis. × 125.



Transverse sections of epidermal and hypodermal tissues: *A*, of densely shaded, noncracking type of Stayman Winesap; *B*, normal fruit of Arkansas, a noncracking variety; *C*, normally exposed but uncracked fruit of Stayman Winesap; *D*, adjacent to a crack in Stayman Winesap. × 300.

photomicrographs of plates 2 and 3, representing varieties resistant to cracking, cells in the hypodermal regions are nearly isodiametric in cross-sectional outline and we may assume that, in these varieties, the rapid expansion necessary in the outermost tissues during the later stages of fruit enlargement was taken care of by cell growth.

Sections of Stayman Winesap tissue adjacent to or including a crack (pls. 1 and 4, *A*) show even greater elongation of the hypodermal cells than was observed in normal tissue of this variety. In many of these cells the lumen is almost closed as a result of tangential stretching or a combination of stretching and radial compression from the swelling inner tissues. Cells such as these appear to have reached their limits of extensibility, and further growth of the fleshy portion of the fruit without commensurate growth in the hypodermis might be expected to sever these tissues and result in a crack. The more rounded hypodermal cells of noncracking varieties might, without further growth but simply by tangential elongation, permit a considerable increase in fruit volume without being torn apart.

TISSUE MODIFICATION IN SUNBURNED, SPRAY-INJURED, AND RUSSETED APPLES

Plate 4, *B* shows tissue of Stayman Winesap in a region characterized by sunburn, a condition of the fruit commonly associated with cracking. The loosely knit structure of the hypodermal layer suggests that growth of the cells in this region ceased or was greatly restricted before growth of the fruit as a whole came to an end, the hypodermal cells, consequently, having been pulled apart. A similar condition, the cause of which is not definitely known, is shown in plate 4, *C*. This apparent injury of the subepidermal layers is thought to be of the same origin as that described by Baker (*1*) and attributed by him to the use of a lime-sulphur fungicide late in the growing season. This condition, like that associated with sunburn, seems to predispose the affected region to cracking. We find here a disintegration of both epidermal and hypodermal tissues that would preclude further growth in these layers. Subsequent expansion in response to continued fruit growth should lead to cracking in an area thus affected.

Tissue modifications that appear in the condition commonly referred to as russeting, and which greatly increase the susceptibility of an apple to cracking, are shown in plate 5. Such russeting, or cork formation, takes place following injury to the cuticle. A layer of cork cambium assumes the position normally occupied by the epidermis, cutting off to the outside several, or many, tangential layers of cork cells, which constitute the scurflike russet. In the sections of russeted fruit that were examined there was little evidence of the formation of phelloderm cells that usually are cut off to the inside by the cork cambium. Beneath the cork tissue the hypodermal cells often were much elongated tangentially (pl. 5, *A*) as in nonrusseted tissues adjacent to or including a crack (pls. 1, 4, *A*, and 6, *D*).

In many sections through russeted areas it was observed that a narrow band of cork tissue extended into and, at some points, beneath the hypodermal layer (pl. 5, *A*, *B*). Hypodermal cells so separated from the cortex often presented a striated appearance owing to tangential stretching, and this tissue was badly disrupted. Apparently growth

had been terminated in this region while the fruit still was enlarging, in consequence of which the cells first were stretched to the limit of their extensibility and then were gradually pulled apart. This would add to the roughness of these russeted areas, and it might lead to incipient cracking as shown in plate 5, *A*.

TISSUE MODIFICATIONS IN DENSELY SHADED STAYMAN WINESAP FRUITS

In 3 years of observation, sound, densely shaded Stayman Winesap fruits growing in the innermost parts of the tree were never found to crack. Upon examination such shaded fruits seemed always to have thinner skins than specimens from more exposed parts of the tree. Apples selected at random in different exposures on several trees and enclosed in brown paper bags from 3 to 4 weeks before harvest seemed also to have abnormally thin skins when they reached maturity, and the incidence of cracking in these bagged apples was much less than in fruits normally exposed. In 1932 there were only 5.2 percent of cracked apples among 210 that had been covered, while among 190 that had been tagged and left exposed, 41.0 percent had cracked.

Zschokke (*13*) states that apples of a variety normally russeted, if shaded early in the growing season, remained free of russet and were thinner skinned than unshaded fruits. Magness and Diehl (*4*) found with several different varieties that the skin on the cheek exposed to the sun was thicker than the skin on the shaded cheek. As shown in plate 6, *A*, sections of tissue of Stayman Winesap apples from densely shaded parts of the tree lack the clearly differentiated hypodermal layer seen in sections of exposed fruits of this variety. The hypodermal cells of these shaded fruits resemble those of varieties that are not susceptible to cracking. They are rounded in outline, they are not clearly distinct from other cells of the cortex, and they lack the stretched appearance of corresponding cells in more exposed specimens of the same variety.

A and *B* of plate 6 show the similarity in structure of the hypodermal regions of a densely shaded Stayman Winesap apple and a normal fruit of Arkansas, a noncracking variety; and these may be contrasted with *C* and *D*, showing, respectively, hypodermal tissues characteristic of normally exposed and of cracked Stayman Winesap apples. The close relationship between degree of tangential stretching of the hypodermal cells and cracking is clearly evident in these illustrations.

Darkly stained cell inclusions, as seen in plate 6, *D*, were observed commonly in tissues adjacent to or including a crack. The nature of these inclusions has not been determined, but it is suggested that they might consist of dead protoplasm and other contents of cells that were killed sometime before the fruit was picked. Upon the death of these cells their growth would, of course, be terminated, with the result that the cells would be elongated and compressed as growth continued in the underlying tissues. Similar inclusions were present in cells of sunburned tissue, as shown in plate 4, *B*, in which the dark intracellular masses are to be seen adhering to the walls of the cells. These cells, largely separated from the subadjacent tissue through which their nourishment had been derived, must surely have died sometime prior to picking of the samples. Had they been elongated and flattened as were those shown in plate 6, *D*, they would have pre-

sented much the same appearance as these, with slitlike lumina filled by the dark inclusions.

Plate 6 also affords a comparison of cell-wall thickness in cracking and noncracking specimens of Stayman Winesap. The much greater thickness of hypodermal cell walls in plate 6, *D* than in 6, *A*, is characteristic of all of the cracked specimens examined except those in which spray injury or russeting were involved. Not only are the cell walls of cracked specimens thicker than those of densely shaded fruits but, as a rule, they are thicker than those of normally exposed but uncracked fruits of the same variety.

The cell walls of hypodermal tissue in normal fruits of Stayman Winesap are not appreciably thicker than in normal fruits of Winesap, which is a variety highly resistant to cracking. We cannot, therefore, regard the thickness of hypodermal cell walls in fruits of Stayman Winesap as an inherent characteristic that bears a causal relationship to cracking. We may, however, consider that the unusual thickness of these cell walls in specimens of Stayman Winesap in which cracking has occurred constitutes a special form of tissue modification to which this variety is especially prone, and which, perhaps, contributes to the tendency toward cracking in such specimens.

DISCUSSION AND CONCLUSIONS

It appears from these observations of tissue structure that the high degree of susceptibility to cracking in Stayman Winesap apples is attributable primarily to a tendency toward marked restriction of growth in the hypodermal layer late in the growing season while the fleshy portions of the fruit may be enlarging at a normal or an excessive rate. Under average conditions, when enlargement of the fruit is proceeding moderately, this restricted growth of the hypodermal cells is supplemented by tangential stretching, and a combination of these two processes (growth and stretching) enables the hypodermal layer to expand sufficiently to keep pace with enlargement of the main body of the fruit. At the same time, the epidermal layer remains intact through a gradual stretching, and increase in quantity, of the cuticle in which it is partially embedded. Thus, when growth proceeds at a moderate rate the fruit seldom cracks; but under conditions of very high humidity and its attendant low transpirational water loss, when tissue hydration is increased and the rate of fruit enlargement is abnormally rapid (*11*), the limit of extensibility of the hypodermal tissue soon is exceeded and cracking occurs. Growth measurements taken at times when Stayman Winesap apples were in the process of cracking have shown (*10*) that many noncracking specimens enlarge at more rapid rates than those that crack. The phenomenon of cracking cannot, therefore, be attributed to an exceptionally rapid rate of swelling in the cortex of cracking specimens, but must be regarded as a result of inadequate growth in their peripheral tissues.

Incipient cracking frequently involves only the cuticle and epidermis, the rupture of which gives rise to cork cambium that soon replaces the normal epidermal covering with less extensible cork tissue, or russet, which predisposes the affected area to more severe cracking at some later time. In deeper cracks the epidermal and hypodermal layers may occasionally behave independently as shown in plate 4, *B* and *C*, where the epidermis remained intact when cells of the hypo-

dermal layer were pulled apart; but it is evident that, as a rule, these two tissues remain closely knit and behave as one in such cracking. To some extent the incapacity to maintain an adequate growth rate in times of abnormal growth acceleration in the deeper tissues seems to include part of the cortex below the hypodermis, since cracks commonly involve the cortex to a depth of 10 or more cells. As previously pointed out, the configuration of an apple is such that expansion of its tissues in a tangential plane during growth must be greater in rate as the issue in question is farther removed from the central axis of the fruit. It is to be expected, therefore, that in times of unusual growth acceleration other regions of the cortex may, like the hypodermis, become involved in cracking by reason of inability to maintain a growth rate commensurate to that of the underlying tissue.

There is a rather common view that cracking indicates lack of strength or resistance on the part of the fruit "skin" to withstand the pressure imposed on it by internal growth. Thus, Tetley (*8*), in discussing cracking in Cox Orange Pippin apples, states that the "epidermis was unable to resist the rapid swelling of the cells within and had consequently cracked." In view of the observations here reported it is doubtful whether we should look upon these outermost tissues of the fruit as offering any considerable mechanical resistance to expansion of the deeper tissues. At best, the epidermal and hypodermal layers combined constitute a covering not exceeding one-fifth of a millimeter in thickness, and in some varieties especially resistant to cracking, such as Arkansas and Winter Banana, no clearly defined hypodermal layer can be distinguished. Cracking, therefore, should be looked upon, not as evidence of failure of these outer tissues to repress an excessive growth rate but rather as evidence of their failure to keep pace with it. In other words, the problem of cracking in the apple involves inability of the peripheral region to stretch or to grow as rapidly as it should when the fleshy portion of the fruit is enlarging at an abnormal rate.

There is, of course, a considerable strain on these outer tissues as growth takes place, and when this strain becomes excessive, as it does when there is inadequate tangential growth or stretching, we may expect these tissues to be torn apart first at their weakest points. It has been observed in many instances that cracks in apparently sound tissue had their origin in hypertrophied lenticels. In lenticel hypertrophy there is a considerable extension of cork tissue beyond the normal limits of the lenticel. According to Eames and MacDaniels (*2*), cork tissue in most plants is both inextensible and inelastic. In an epidermis under great strain because of disproportionate growth of epidermal and cortical tissues such localized areas of inextensible cork would mark points at which the tissues might first be pulled apart. This role of lenticels in cracking of apples probably is not of great importance, however, if we assume that the antecedent cause of cracking lies in failure of the peripheral tissues as a whole to maintain an adequate rate of growth. In the absence of lenticels cracks might occur almost as soon in uninterrupted tissue. No data are available to show the percentage of cracks that originate in hypertrophied lenticels, but observation has shown that cracks frequently appear without relation to them.

The high incidence of cracking in parts of the apple affected by russeting, sunburn, scab lesions, and other skin injuries can be attributed directly to these abnormalities. Such modifications of the fruit periphery involve considerable areas in which extensibility of the tissues is below normal. This is demonstrated by the photomicrographs of sunburned, spray-injured, and russeted tissues in plates 4, *B* and *C*, and 5, respectively. In the first two instances the capacity for explansion of most of the tissue comprising the fruit skin has been prematurely halted by death of many cells in this region. In the case of russeted areas the tendency to crack has been aggravated by partial disruption of the hypodermal layer or by substitution of inextensible cork for the normal epidermal and subepidermal tissues. Freezing-point depressions of the cortex underlying these modified skin areas have been shown (*10*) to be appreciably higher than under normal skin. Thus, abnormal tissue pressures in the cortex combine with subnormal extensibility of the peripheral layers of cells in making these sunburned, russeted, and otherwise injured portions of the apple especially prone to crack.

We can only speculate as to the probable causes of the structural peculiarities that render exposed fruits of a susceptible variety of apple more likely to crack than others. It is known that exposure of plant tissues to direct sunlight increases transpirational water loss. Fruits most exposed to sunlight also are most exposed to wind and general air movement, which would tend further to remove water from their tissues. Cell growth, which can take place only when the cell is turgid, might thus be checked in periods of intense sunshine and high evaporativity, owing to a water-saturation deficit of the tissues. According to Palladin (*5*) a severe water deficit promotes thickening of cell walls in the affected region. Thus, the premature cessation of cell growth and abnormal thickening of cell walls associated with cracking in exposed parts of Stayman Winesap apples might be considered to result from the drying effects of their immediate environment. Under conditions of prolonged hot, dry weather, especially when accompanied by soil-moisture deficiency, all of the fruits on a tree might be affected in a similar manner, but in various degrees, as a result of inadequate water supply to the outer layers of cells. Such a hypothesis would serve to explain the common observation that cracking is most likely to occur and affects the largest percentage of the crop if an abundant moisture supply is made available to the fruit following a protracted period of water shortage.

Cessation or restriction of growth in hypodermal tissues underlying russeted areas (pl. 5, *A*), might be due to a prolonged water deficit resulting from excessive moisture loss through the overlying zone of cork, which, according to Baker (*1*), is much more pervious to water than is normal cuticle. When all or part of the hypodermal region is separated from the cortex and from contact with vascular elements by a layer of cork, as in plate 5, *B*, further growth of the hypodermal cells might be inhibited by reason of restricted passage of nutrients and water through the suberin-impregnated zone below them. Occasionally both the epidermal and the subepidermal tissues are completely replaced by cork. In all of these conditions associated with russeting the normal peripheral tissues have been replaced by tissues that are much less extensible and that, therefore, increase the susceptibility of the affected area to cracking.

There is no evidence indicating that the theory of Kertesz and Nebel (*3*) regarding the role of colloids in cracking of cherries is applicable to this phenomenon in apples. In all of the varieties studied the smallest cells and the thickest cell walls (therefore, the greatest proportion of colloidal cell-wall materials) were found in the outermost tissues of the fruit. Upon excessive absorption of water in this region a condition should be approached just the reverse of that which causes cracking; that is, there should be expansion of the peripheral tissues in excess of the requirements imposed upon these tissues by growth of the deeper-lying cortex. In the cortex, where an abnormal rate of swelling by absorption of water seems responsible for the tissue strain that promotes cracking, the proportion of cell-wall material to osmotically active cell contents is much smaller than in the outer tissues, because of the larger cell sizes and thinner cell walls in the cortex.

Although some water may be absorbed through the skin of Stayman Winesap apples in periods of rain (*10*), most of the water supply permeating the fruit in times of cracking seems to enter through the usual channels of the vascular system. If the cell walls of the cortex were able to imbibe this water and swell appreciably before the peripheral tissues had been able to expand proportionately by the same process, we might expect cracking to occur in somewhat the manner suggested by Kertesz and Nebel (*3*) for cherries. However, there is no reason for supposing that the deeper cortical tissues would have access to an increased flow of water in the vascular system very long before it reached the hypodermal layer, where, as pointed out, colloidal absorption and swelling would tend to prevent rather than to promote cracking. It has been shown (*10*) that detached Stayman Winesap apples, with stems and calyxes sealed with paraffin, may crack severely when they are held for a period of 48 hours under water. Thus, even when an increased water supply is made available first to the small, thick-walled cells of the outer tissues by absorption directly through the skin, relatively greater swelling takes place in the region of the larger, thinner-walled cells of the cortex, and cracking results.

There seems to be little basis for assuming that the colloidal constituents of the cell walls would play a more important part in swelling of the fruit than do the osmotically active constituents of the cell vacuoles. As pointed out by Shull (*6*), cell solutes and the colloids of the cell wall are approximately in equilibrium with respect to the degree in which their water-saturation deficits are satisfied. Therefore the water-absorbing powers of the cell colloids should not appreciably exceed the osmotic value of the cell solutes at any given time. Although, as Kertesz and Nebel (*3*) point out, some colloids may exert an imbibitional force of as much as 2,000 atmospheres, such high values apply to colloids in a dry, or nearly dry state, whereas in the flesh of a fruit such as cherry or apple the actual colloidal pressure must be only a small fraction of its potential pressure due to the degree of hydration that exists in such succulent tissues. It is more likely that the excessive swelling of the fleshy portion of the fruit in times of abnormal water supply is due to the combined hydration forces of all those constituents of the cell and cell wall that tend to draw water to themselves, including both colloidal substances and cell solutes, which are component parts of the same equilibrated system.

SUMMARY

Different varieties of apple, and different individual fruits within a variety, exhibit striking differences in tendency to crack under the same environmental conditions. In an attempt to find a histological basis for the explanation of such differences fruit tissues of Stayman Winesap, a variety highly susceptible to cracking, and comparable tissues of five varieties usually immune, were sectioned and stained for microscopic study.

In most of the sections of Stayman Winesap that were examined, and especially in tissues in which cracking already had occurred and in those characterized by certain abnormalities commonly associated with cracking, there was definite evidence of premature cessation or retardation of growth in the peripheral tissues. In the epidermis the cells had been separated into small groups and the intervening spaces were filled with cutin, which thus preserved the continuity of the epidermal layer. This condition of the epidermis was evident in both cracking and noncracking varieties, however, and the structure and growth habit of the epidermis are not considered of great importance in determining the degree of susceptibility of a variety to cracking.

In Stayman Winesap the hypodermal layer of cells likewise showed clear evidence of an inadequate growth rate late in the season, as these cells were much elongated tangentially, suggesting that recent enlargement of the fleshy portions of the fruit had been accommodated in the hypodermis primarily by tangential stretching. In noncracking varieties the cells of the hypodermis were nearly isodiametric in cross-sectional outline, and evidently had maintained a growth rate proportional to that of the underlying tissues.

On the basis of these investigations there is advanced a hypothesis that the susceptibility of Stayman Winesap apples to cracking is due chiefly to premature cessation or restriction of growth in the hypodermal layer. Upon unusual acceleration of growth in the fruit cortex, as under conditious of very high atmospheric humidity with its attendant increase in tissue hydration, the limit of extensibility of the hypodermal layer soon is reached and further expansion of the tissues beneath leads to cracking. The phenomenon of cracking, therefore, probably should be regarded, not as a result of failure of the peripheral fruit tissues to repress an excessive growth rate of the cortex but rather as a result of their failure to keep pace with it.

This premature retardation of growth in the hypodermal layer seems to be related in some manner to exposure of the fruit to sun and general air movement, as the condition is greatly accentuated in tissue so exposed and is virtually absent in tissues of densely shaded fruits. Abnormal susceptibility to cracking in sunburned, spray-injured, and russeted areas is due to special types of structural weakness in the parts involved.

LITERATURE CITED

(1) BAKER, CLARENCE E.
1931. A STUDY OF THE SKIN STRUCTURE OF THE GRIMES APPLE AS AFFECTED BY DIFFERENT TYPES OF INJURY. Amer. Soc. Hort. Sci. Proc. (1930) 27:75–81, illus.

(2) EAMES, ARTHUR J., and MACDANIELS, LAWRENCE H.
1925. AN INTRODUCTION TO PLANT ANATOMY. Ed. 1, 364 pp., illus. New York.

111469—38——3

(3) KERTESZ, ZOLTAN I., and NEBEL, B. R.
1935. OBSERVATIONS ON THE CRACKING OF CHERRIES. Plant Physiol. 10: 763–772, illus.
(4) MAGNESS, J. R., and DIEHL, H. C.
1924. PHYSIOLOGICAL STUDIES ON APPLES IN STORAGE. Jour. Agr. Research 27: 1–38, illus.
(5) PALLADIN, V. I.
1923. PLANT PHYSIOLOGY. Authorized English Transl. . . . by Burton Edward Livingston. Amer. Ed. 2, 360 pp., illus. Philadelphia.
(6) SHULL, CHARLES A.
1924. IMBIBITION IN RELATION TO ABSORPTION AND TRANSPORTATION OF WATER IN PLANTS. Ecology 5: 230–240.
(7) SORAUER, PAUL.
1922. MANUAL OF PLANT DISEASES. . . . Transl. by Frances Dorrance. 908 pp., illus. [Wilkes-Barre, Pa.]
(8) TETLEY, URSULA.
1930. A STUDY OF THE ANATOMICAL DEVELOPMENT OF THE APPLE AND SOME OBSERVATIONS ON THE "PECTIC CONSTITUENTS" OF THE CELL-WALLS. Jour. Pomology and Hort. Sci. 8: 153–172, illus.
(9) TUCKER, LOWELL R.
1934. A VARIETAL STUDY OF THE SUSCEPTIBILITY OF SWEET CHERRIES TO CRACKING. Idaho Agr. Expt. Sta. Bull. 211, 19 pp., illus.
(10) VERNER, LEIF.
1935. A PHYSIOLOGICAL STUDY OF CRACKING IN STAYMAN WINESAP APPLES. Jour. Agr. Research 51: 191–222, illus.
(11) ———
1937. A STUDY OF GROWTH RATES IN STAYMAN WINESAP APPLES. Amer. Soc. Hort. Sci. Proc. 35: 128–131, illus.
(12) ——— and BLODGETT, E. C.
1931. PHYSIOLOGICAL STUDIES OF THE CRACKING OF SWEET CHERRIES. A PRELIMINARY REPORT. Idaho Agr. Expt. Sta. Bul. 184, 15 pp., illus.
(13) ZSCHOKKE, A.
1897. UEBER DEN BAU DER HAUT UND DIE URSACHEN DER VERSCHIEDENEN HALTBARKEIT UNSERER KERNOBSTFRÜCHTE. Landw. Jahrb. Schweiz 11: 153–197, illus.

PHYSIOLOGICAL CONDITIONS WHICH PRODUCE WING DEVELOPMENT IN THE PEA APHID[1]

By CARL W. SCHAEFER

Instructor in economic entomology, Wisconsin Agricultural Experiment Station

INTRODUCTION

An investigation was conducted during 1935 and 1936, from which data are presented that seem to clear up the existing confusion in regard to the appearance of wings among aphids, and to show more precisely the influence of the physiological "water balance" within aphids upon this phenomenon.

Various theories have been advanced to account for the presence or absence of wings among aphids, yet none of them has been entirely satisfactory. Production of wings has been associated with such epigenetic factors as temperature (*20*, *19*),[2] light (*16*, *24*), starvation (*9*, *10*), wilting of the host (*15*), crowding (*35*), and chemical composition of the host plant (*21*). The appearance of large numbers of alate aphids on wilted and crowded plants, either in early spring, midsummer, or under greenhouse conditions when the environments are different, suggests that climatic conditions are not the primary cause of wing development. The appearance of winged forms among progeny of aphids removed from plants and starved, indicates that unfavorable conditions of the host are only secondary influences on some physiological condition of the aphid. Recently it has been suggested by Ackerman (*1*) and Rivnay (*23*) that the physiological conditions which influence the wing formation are determined by the "water balance" within the aphid.

Correlation of moisture conditions and wing production are evident in the association of wilting of the host plant and starvation of its aphid population. However, the results of all investigators do not agree on this point. The first part of this investigation is devoted to experiments to show the probable reasons for these conflicting results, while the second part is devoted to a presentation of direct evidence relative to wing production, obtained from chemical, anatomical, and biological studies of the aphid.

METHODS

The pea aphid, *Macrosiphum pisi* (Kalt) was used in this investigation. Experiments designed to show the effect of aphid population upon appearance of winged forms were started with apterous fourth-stage individuals from parents which previously had fed on young succulent pea seedlings (*Pisum sativum* var. Alaska). These aphids were placed on experimental pea seedlings which were separated into the following height classes: 2 to 3 inches, 3 to 4 inches, and 5 to 6 inches. Three aphid populations obtained by placing 6, 12, and 20 adults on a plant, were tested on individual plants of the three

[1] Received for publication March 17, 1938; issued December 1938.

[2] Italic numbers in parentheses refer to Literature Cited, p. 839.

height classes. These adults were allowed to reproduce on the plants up to 48 hours, at which time they were removed. Their progeny were allowed to remain on the plants until all had reached the third instar because this stage is the first in which the alate and apterous condition can be identified easily.

The pea seedlings used in the experiments were sprouted in sawdust and then transferred to Erlenmeyer flasks containing a complete nutrient solution. This solution contained 35.4 mg $Ca(NO_3)_2$, 20.4 mg KH_2PO_4, 18.5 mg $MgSO_4$. $7H_2O$, and a trace of $FeCl_3$. $6H_2O$ in 120 cc of distilled water.

The tops of these flasks were squeezed through 1-inch holes spaced 4 inches apart in strips of stiff linoleum 4 inches wide and 15 inches long. Care was taken to have the black surface of the linoleum upward and to have the holes small enough to hold the flask snugly by the rim. The linoleum was supported by two 15-inch laths placed under it against the necks of the flasks and at right angles to the supporting two by fours (fig. 1). Lantern globes placed on the flat

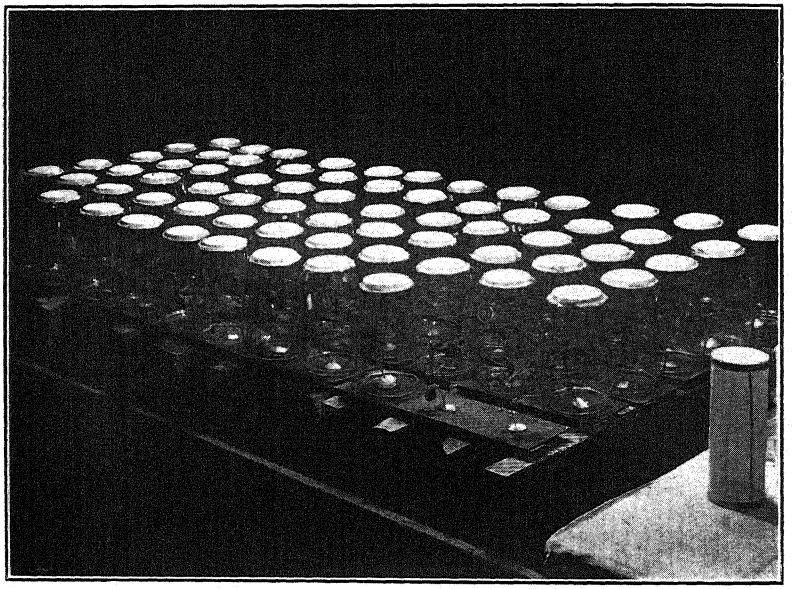


FIGURE 1.—Lantern-globe cages and working bench employed in the investigation.

surface of the linoleum made aphid-tight enclosures around the plants. Winged and wingless aphids, when shaken from the plants, fell onto the black surface of the linoleum where they were separated and quickly counted.

The starvation experiments were started with fourth-stage and adult apterous forms of apterous parentage. Some of them were caged on wilted plants and others were isolated in cheesecloth cages.

For quantitative analyses, large numbers of aphids were collected in alfalfa and pea fields by the sweeping method. Immediately after the aphids were collected, they were separated into the following

classes: (1) First- and second-stage nymphs; (2) fourth-stage apterous forms; (3) fourth-stage alate forms; (4) adult apterous forms; and (5) adult alate forms. Sorting of the aphids was best accomplished by dumping them onto the polished surface of a black photographic squeegee board. Aphids crawling out from the center of the board were separated and sucked into vials with the aid of a rubber tube. They were placed in air-tight rubber-stoppered vials in order that the moisture given up by the aphids in transit to the laboratory might be weighed and included in the determinations.

After each sweeping, a number of aphids in each stage were placed on balsam on a glass slide. A small amount of xylol and a cover glass were dropped over them, and the slides were taken to the laboratory where they were examined under the microscope. All aphids prepared in this manner and all unprepared specimens examined were identified as *Macrosiphum pisi*.

Collections from alfalfa (*Medicago sativa* L.) were made on May 7, 13, and 21 and those from peas on June 12 and 13, 1936. A sufficient number of stem mothers and their nymphs, apterous first-generation adults, and a large number of fourth-stage and adult alate individuals were collected on alfalfa. On peas a sufficient number of fourth-stage apterous forms was available, but only a very few alate adults. So, from both sources a sufficient number of the different forms was obtained for comparing the quantitative compositions.

Live weights of the aphids were obtained by subtracting the weights of the empty airtight rubber-stoppered vials at room temperature from the weights of the vials with aphids. Dry weights were obtained in a like manner after the vials had been 48 hours in a vacuum oven. A known dry weight of aphids was then placed in Whatman's No. 40 double acid-washed filter paper, put in an extraction thimble in a Caldwell extracting tube, and extracted with anhydrous ether in the usual manner.

The protein content of fat-free aphids was determined by the macro-Kjeldahl (*12*) and semimicro-Kjeldahl (*30*) methods.

Sugar analysis was determined by Elser's titration method (*7*). A known weight of 50 to 100 mg of fat-free aphids were weighed into a 50-cc beaker. About 4 cc of warm water was added and in this the aphids were macerated. The beaker with the aphid solution was kept for the next 12 hours between 35° and 40° F. and the total volume was then brought to 10 cc in a volumetric flask and 3-cc aliquots were examined in the usual manner for invert and higher sugars.

Serial sections of alate and apterous aphids were obtained by the paraffin method of preparation. Microdissections were made on aphids embedded in paraffin under water.

The volume of honeydew excreted by the pea aphid was measured by counting the number of drops expelled and obtaining their diameter under a stage micrometer. The drops were collected on sheets of cellophane which were placed daily inside the regular cages in which the aphids were confined.

MAXIMUM PRODUCTION OF ALATE PROGENY AND CONDITIONS WHICH INDUCE IT

Investigators generally agree that winged parents give birth to wingless progeny, (*33*, *25*), but all the results reported regarding the appearance of winged progeny of apterous parents do not agree. Kyber (*15*), Walker (*32*), Gentry (*8*), Mordwilko (*18*), and Davis and Whitehead (*6*) associated the appearance of winged forms with "underfeeding" of the aphids. Slingerland (*26*) reported that lack of food or crowding produced dwarfed apterous forms. Tannreuther (*29*) and Börner (*2*) associated the appearance of winged forms with abundance of food. Davidson (*4*) and Mason (*17*) found no increase of alate individuals on unsatisfactory food.

The probable explanation of these conflicting reports is partly shown by a simple experiment in which plants of three arbitrarily chosen heighth classes, were used each with three arbitrarily selected populations. The results of this experiment are given in table 1 and shown graphically in figure 2. These results indicate not only

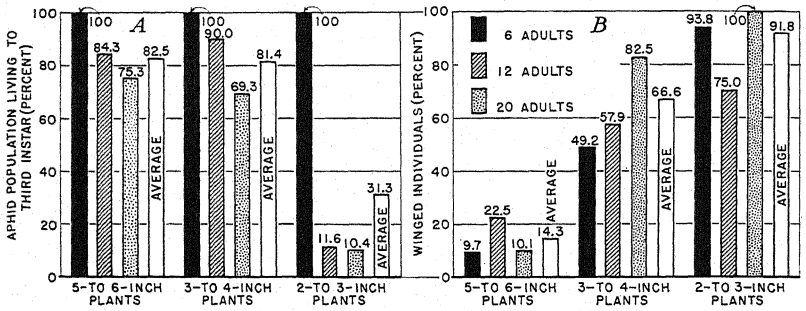


FIGURE 2.—Relation of ability of plants to support populations of aphids (*A*) and appearance of winged individuals (*B*).

that the ability of a pea plant to support an aphid population tends to decrease as the size of the plant decreases and as the size of the population increases, but also that the number of winged individuals tends to increase as the population increases, as the ability of the plant to support populations decreases, and as the size of the plant decreases. The correlation between appearance of winged forms and size of population is not so striking as the correlation between the appearance of winged forms and the size of plant. Tables 2 and 3 likewise show this lack of uniformity. However, these results may be explained by the possible variation in the physiological conditions of the plant or the aphids.

TABLE 1.—*The effect of size of population and of host upon the ability of the plant to support populations of pea aphids, and upon the appearance of winged forms*

Height of plants, and period adults were on them	Item	Data for populations produced by placing indicated number of adults on plants		
		6	12	20
5 to 6 inches; 48 hours	Adults—number	24	48	80
	Total progeny—do	321	631	935
	Average progeny per plant—do	80.2	157.8	233.8
	Progeny reaching third instar—do	321	532	704
	Average progeny per plant to 3d instar—do	80.2	133.0	176.0
	Progeny reaching third instar—percent	100.0	84.3	75.3
	Alate—number	31	120	71
	—do—percent	9.7	22.5	10.1
3 to 4 inches; 24 hours	Adults—number	36	72	120
	Total progeny—do	260	462	729
	Average progeny per plant—do	43.3	77.0	121.5
	Progeny reaching third instar—do	260	416	505
	Average progeny per plant to third instar—do	43.3	69.3	84.2
	Progeny reaching third instar—percent	100.0	90.0	69.3
	Alate—number	128	241	417
	—do—percent	49.2	57.9	82.5
2 to 3 inches; 20 hours	Adults—number	23	37	61
	Total progeny—do	160	275	268
	Average progeny per plant—do	40.0	68.8	67.0
	Progeny reaching third instar—do	160	32	28
	Average progeny per plant to third instar—do	40.0	8.0	7.0
	Progeny reaching third instar—percent	100	11.6	10.4
	Alate—number	150	24	28
	—do—percent	93.8	75.0	100.0

The foregoing experiment was modified to show the effect of previous nutrition of parent aphids on the production of alate offspring. Aphids were allowed to remain on test plants from 20 to 48 hours, and then transferred to a set of comparable plants which had not previously been infested. The results for the 2- to 3-inch plants and the 3- to 4-inch plants are given in tables 2 and 3. Aphids which fed on the 2- to 3-inch plants with one exception (table 2) and which had formerly given birth to large numbers of alate forms failed to maintain their previous ratio of alate offspring. Those aphids on the larger plants with one exception (table 3) increased their ratio of alate offspring.

These results support the contention of Kyber (*15*) that underfeeding is associated with the appearance of wings, provided the parent aphids have previously been on succulent food. They also support the contention of Slingerland (*26*) that underfeeding is associated with the appearance of "dwarfed" apterous forms, provided the parent aphids have previously been subjected to poor feeding conditions and have given birth to considerable numbers of winged forms. In one case an abundance of winged forms was obtained while in the other a larger number of apterous forms resulted.

TABLE 2.—*Effect of size of population on the ability of plants to support pea aphids and on the appearance of winged forms, compared on plants 2 to 3 inches in height during consecutive periods of 20, 24, and 36 hours*

Reproduction period of adults on plants	Item	Data for populations produced by placing indicated number of adults on 4 plants		
		6	12	20
First 20 hours	Adults—number	23	37	61
	Total progeny—do	160	275	268
	Progeny reaching third instar—do	160	32	28
	Average progeny per adult—do	7.0	.9	.5
	Average progeny per plant to third instar—number	40.0	8.0	7.0
	Alate—do	150	24	28
	—do—percent	93.8	75.0	100.0
Next 24 hours	Adults—number	23	24	61
	Total progeny—do	139	169	233
	Progeny reaching third instar—do	139	133	122
	Average progeny per adult—do	6.0	5.5	2.0
	Average progeny per plant—do	34.8	33.2	30.5
	Alate—do	129	107	114
	—do—percent	92.8	80.4	93.4
Next 36 hours	Adults—number	18	24	37
	Total progeny—do	172	145	313
	Progeny reaching third instar—do	172	67	118
	Average progeny per adult—do	9.5	2.8	3.2
	Average progeny per plant—do	43.0	16.8	29.5
	Alate—do	116	28	88
	—do—percent	67.4	41.8	74.6
Total	Adults—number	64	85	159
	Total progeny—do	471	589	814
	Progeny reaching third instar—do	471	232	268
	Average progeny per adult—do	7.4	2.7	1.7
	Average progeny per plant—do	39.2	19.3	22.3
	Alate—do	395	159	230
	—do—percent	83.9	68.5	85.8

TABLE 3.—*Effect of size of population on the ability of plants to support pea aphids and on the appearance of winged forms, compared on plants 5 to 6 inches in height during 4 consecutive 48-hour periods*

48-hour period adults were on plants	Item	Data for populations produced by placing indicated number of adults on 4 plants		
		6	12	20
First	Adults—number	24	48	80
	Total progeny—do	321	631	935
	Progeny reaching third instar—do	321	532	704
	Average progeny per adult—do	13.4	11.1	8.8
	Average progeny per plant—do	80.2	138.0	176.0
	Alate—do	31	120	71
	—do—percent	9.7	22.5	10.1
Second	Adults—number	24	47	79
	Total progeny—do	318	622	888
	Progeny reaching third instar—do	318	618	786
	Average progeny per adult—do	13.3	13.1	9.9
	Average progeny per plant—do	79.5	154.5	196.5
	Alate—do	1	206	260
	—do—percent	.3	33.3	33.1
Third	Adults—number	23	[1] 36	79
	Total progeny—do	290	442	771
	Progeny reaching third instar—do	290	442	768
	Average progeny per adult—do	12.6	12.3	9.7
	Average progeny per plant—do	72.5	110.5	192.0
	Alate—do	1	189	293
	—do—percent	.3	42.8	38.1
Fourth	Adults—number	22	46	76
	Total progeny—do	299	622	894
	Progeny reaching third instar—do	299	622	894
	Average progeny per adult—do	13.6	13.5	11.8
	Average progeny per plant—do	74.8	155.5	223.
	Alate—do	9	136	306
	—do—percent	3.0	21.8	34.2

[1] Some young of 11 adults on 1 plant escaped, therefore only data for 36 adults were included here.

RESULTS OF STARVING APTEROUS PARENTS IN THE FOURTH INSTAR AND IN THE ADULT STAGE ON SUBSEQUENT PRODUCTION OF ALATE PROGENY

Winged offspring appear among the progeny of starving apterous parents and of apterous parents placed on wilted plants, according to the results of Göldi (*9*), Gregory (*10*), Wadley (*31*), Ackerman (*1*), Reinhard (*22*), Shull (*25*), and others. This phenomenon also was noted in preliminary observations in this investigation. However, it was discovered that when fourth-stage aphids were starved or placed on wilted or semiwilted plants, few alate individuals appeared among the progeny (table 4).

TABLE 4.—*Difference in appearance of winged forms among the progeny of pea aphids starved in the fourth stage and of those starved in the adult stage*

[Wilted and semiwilted plants used]

Item	Fourth-stage aphids	Adult aphids
Adults.....number..	80	80
Progeny reaching third instar.....do....	153	247
Alate.....do....	5	81
Do.....percent..	3.3	32.8

This experiment was repeated with (1) aphids that were kept constantly on succulent pea seedlings as a check, (2) aphids that were removed from the succulent food for a period of 12 hours and then returned to their food plants, and (3) aphids that were removed for a period of 24 hours and then replaced on the plants. Aphids in group 1 were weighed when the experiment was started. All aphids of the latter two groups were weighed when they were removed and also when they were replaced. After they were returned to the plants they were allowed to reproduce for a period of 46 hours; then transferred to another group of plants and left for a period of 15 hours. Again they were transferred and left on a third group of plants for a period of 10 hours.

The results of this experiment are given in table 5. As in the previous experiment, the starved adults produced a higher proportion of alate progeny than those starved in the fourth stage. In addition, the experiment substantiates the view of Klodnitski (*13*) that the winged "anlage" are checked when prospective alate-producing adults are replaced on succulent growth.

TABLE 5.—*Difference in appearance of winged forms among the progeny of pea aphids starved in the fourth stage and of those starved in the adult stage*

Weight of aphids and period elapsed	Item	Fourth-stage aphids			Adult aphids		
		Not starved	Starved 12 hours	Starved 24 hours	Not starved	Starved 12 hours	Starved 24 hours
Average weight before starving...milligrams		1.71	1.70	1.70	2.44	2.29	2.41
Average weight before placing on plants...do		1.71	1.28	1.26	2.44	1.96	1.88
First 46 hours	Adults...number	10	8	6	10	8	7
	Progeny reaching third instar...number	37	8	3	117	49	42
	Alate...do	3	0	0	4	15	28
	...do...percent	8.1	0	0	3.4	30.6	66.7
Next 15 hours	Adults...number	10	4	5	10	5	5
	Progeny reaching third instar...number	60	16	30	85	43	29
	Alate...do	4	1	0	0	1	6
	...do...percent	6.7	6.3	0	0	2.3	20.7
Next 10 hours	Adults...number	6	3	4	10	5	5
	Progeny reaching third instar...number	31	15	26	56	30	23
	Alate...do	1	0	0	0	0	2
	...do...percent	3.2	0	0	0	0	8.7
Total	Adults...number	26	15	15	30	18	17
	Progency reaching third instar...number	128	39	59	258	122	94
	Alate...do	8	1	0	4	16	36
	...do...percent	6.2	2.6	0	1.6	13.1	38.3

QUANTITATIVE CHEMICAL ANALYSES OF ALATE AND APTEROUS APHIDS

Such phenomena as the lack of winged forms among the progeny of winged parents and of apterous parents subjected to unfavorable conditions in the fourth stage, and the inability to continue maximum production of winged forms, indicates that the controlling wing-producing mechanism is influenced by an accumulating and diminishing process of some sort. Ackerman (*1*) recognized the presence of such a changing process, and from a study of fat globules he concluded that the appearance of winged forms of the grain aphid was "dependent upon changes in the proportion or concentration of certain materials in the haemolymph as brought to pass by the rupture of the brown globules" (*1*, *p. 59*).

When the pea aphid was examined for type of globule content, there were found a large number of small, greenish, "plastidlike" structures that appeared to be fatty in nature, small clear globules and large clear globules, but no distinct brown globules.

Under close examination the number of globules, both the green and the clear, seemed to vary in the different aphids examined. This apparent difference prompted a quantitative analysis of different forms of the aphid. The results of the analysis are shown in tables 6 and 7. Differences in fat and moisture content are greater than differences in protein and sugar content. The fat content of the winged forms is greater than that of the apterous forms and the moisture content is smaller. Nymphs have a greater fat content than their mothers, but there is little difference in the moisture content of the two forms.

TABLE 6.—*Moisture, fat, and protein content of aphids collected from alfalfa and from peas in 1936*

Plant and stage of aphid	Moisture determinations				Ether-extract determinations				Protein determinations			
	Date collected	Live weight	Dry weight	Moisture content	Dry weight	Weight of ether extract	Fat content		Weight of fat-free residue	Weight of protein in fat-free residue	Protein content	
							Dry-weight basis	Live-weight basis			Dry-weight basis	Live-weight basis
		Milligrams	*Milligrams*	*Percent*	*Milligrams*	*Milligrams*	*Percent*	*Percent*	*Milligrams*	*Milligrams*	*Percent*	*Percent*
Alfalfa:												
Stem mothers	May 7	714.0	145.9	79.6	145.9	15.4	10.6	2.2				
	do	788.6	154.8	80.4	154.8	17.3	11.2	2.2	137.5	80.7	52.1	10.2
Nymph of stem mothers	do	367.6	78.7	78.6	78.6	10.6	13.5	2.9				
	do	433.2	93.5	78.4	93.5	13.1	14.0	3.0	80.6	34.6	36.9	8.0
Alate fourth instar	May 13	705.8	187.1	73.5	90.5	23.5	25.9	6.9	63.1	30.9	36.2	9.6
	May 21	324.0	84.5	73.9	226.3	57.7	25.5	6.7	109.1	53.5	36.5	9.6
Alate adult	May 13	565.7	148.0	73.8	113.6	29.0	25.5	6.7	80.2	44.5	41.3	10.8
	May 21	138.0	36.4	73.6	122.6	32.1	26.2	6.9	57.1	33.6	43.5	11.5
Apterous adult	May 13	927.6	207.7	77.6	96.8	14.6	15.1	3.4	156.4	86.6	47.0	10.5
	May 21	151.4	33.5	77.9	131.5	20.9	15.9	3.5	69.9	36.7	44.2	9.8
Peas:												
Nymphs	June 12	369.5	81.9	77.8	233.5	52.6	22.5	5.0	27.3	17.2	48.8	10.8
	June 13	197.7	42.8	78.3	180.6	43.3	23.9	5.2	27.5	17.3	47.8	10.4
Alate fourth instar	June 12	210.7	55.6	73.6	256.0	90.0	35.1	9.3	29.6	17.9	39.3	10.4
	June 13	159.6	42.1	73.6	162.7	54.5	33.5	8.8	29.2	17.3	39.4	10.4
Apterous fourth instar	June 12	251.9	56.7	77.5	220.0	52.4	23.8	5.4	29.2	16.3	42.5	9.6
	June 13	282.0	61.4	78.2	196.4	43.4	22.1	4.8	27.7	16.5	46.4	10.1
Apterous adult	June 12	489.7	111.1	77.3	388.0	81.7	21.1	4.8	30.5	18.4	47.6	10.8
	June 13	371.4	81.8	78.0	307.1	63.6	20.7	4.6	29.1	18.6	50.7	11.2

TABLE 7.—*Sugar content of aphids collected from alfalfa and peas in 1936*

Stage of aphid	Alfalfa							Peas						
	Date collected	Weight of fat-free sample	Invert sugar		Dextrin		Total sugar on live-weight basis	Date collected	Weight of fat-free sample	Invert sugar		Dextrin		Total sugar on live-weight basis
			Weight	On live-weight basis	Weight	On live-weight basis				Weight	On live-weight basis	Weight	On live-weight basis	
		Milligrams	*Milligrams*	*Percent*	*Milligrams*	*Percent*	*Percent*		*Milligrams*	*Milligrams*	*Percent*	*Milligrams*	*Percent*	*Percent*
Nymphs								June 12	36.2	1.9	0.9	1.6	0.8	1.7
								June 13	79.3	3.5	.8	5.1	1.1	1.8
Alate fourths	May 13	71.4	5.5	0.8	7.6	1.0	1.8	June 12	60.4	2.5	.8	3.1	.9	1.6
	May 21	56.6	3.6	1.2	1.7	.6	1.8	June 13	56.4	2.5	.8	2.5	.8	1.6
	do	61.5	1.3	1.4	2.7	.8	2.2	do	43.0	2.7	1.1	2.3	.7	1.7
Alate adult	May 13	62.9	3.6	1.1	2.3	.8	1.9							
	May 21	44.5	2.8	1.1	2.8	1.1	2.2							
Apterous fourth instar								June 12	81.2	3.1	.6	4.8	1.0	1.7
								June 13	76.3	2.9	.6	4.6	1.1	1.7
Apterous adult	May 13	77.6	6.2	1.5	4.4	1.1	2.6	June 12	106.5	3.5	.6	5.4	.9	1.5
	May 21	64.4	5.6	1.5	3.7	1.0	2.6	June 13	106.8	2.7	.5	3.8	.6	1.1
	do	119.5	9.2	1.3	5.5	.8	2.1	do	93.5	2.9	.5	4.1	.8	1.4

The protein content of adults is greater than that of immature forms and the protein content of alate forms is greater than that of apterous forms (table 6).

The sugar content of aphids collected from peas is noticeably lower than that of aphids collected from alfalfa. Apterous adults collected from alfalfa have a greater sugar content than all other forms collected on alfalfa, but apterous adults collected from peas have a lower sugar content than all other forms collected from peas.

The greater fat content of alate forms probably constitutes a source of reserve energy for use during migration. Nymphs have a greater fat content than their mothers probably because of the presence among them of a number that are destined to become alate. Apterous and alate adults might have a greater protein content than their nymphs because of the presence of developing young in their abdomens. Alate forms might have a greater protein content because of the presence of large wing-propelling muscles. Differences in the sugar content of aphids collected from alfalfa and those collected from peas are not well understood, but it may be that aphids feeding on peas take in more fluid than those feeding upon the less succulent alfalfa. As more fluid is ingested more will be egested and there will be a better opportunity to void the unnecessary sugars.

FOOD SOURCE, NATURE OF FOOD, AND STRUCTURE OF THE DIGESTIVE TRACT OF APHIDS

Aphids are known to penetrate to the phloem vessels (*5*, *11*, *27*, *28*). Davidson (*5*, *p. 51*) observed that—

> Aphids appear to feed almost continuously and large quantities of soluble carbohydrates are taken up in the cell sap. As these insects do not possess Malpighian tubules, the waste products, including surplus sugars, are passed out through the anus.

The Alimentary tract of the pea aphid was found to possess features similar to those of other aphids. Except for size and slight differences in arrangement, the digestive tract resembled that described for *Schizoneura lanigera* by Davidson (*3*). The entire length of the large intestine lies proximal to the stomach but does not lie partly enclosed in the stomach epithelium as was described by Knowlton (*14*) for *Longistigma caryae*. In some cases the stomach and large intestine were held together by tracheae, but these were easily pulled away. The cross sections show that the small intestine possesses a reduced lumen having the form of a T. The large cells of the thick intestinal wall contain large nuclei. The presence of these large nuclei indicate a probable excretory function. No malpighian tubules were found.

The passage of the excess fluid through the body of the aphid is summarized by Wigglesworth (*34*, *p. 45*) as follows:

> But instead of the superfluous fluid being taken into the blood and then eliminated by the Malpighian tubes in the manner just described [for blood-sucking forms], a dilated loop of the fore-gut, the "filter chamber," with very delicate walls, is invaginated into the rectum; the unwanted fluids are here absorbed, or perhaps simply filtered, directly into the terminal part of the hind-gut; and so come to be discharged again to the exterior as honey dew, manna, and such-like products.

Aphids must take in large quantities of plant sap in order to obtain the supply of nitrogenous food that is necessary for rapid reproduction. This sap is mostly moisture, and since a large amount of unnecessary carbohydrates are in solution, the problem becomes one of quickly voiding the liquid and the unnecessary waste products. The structure found in aphids apparently satisfies the requirements for rapid elimination of moisture and carbohydrates.

VOLUME OF HONEYDEW EXCRETED BY THE PEA APHID

Aphids appear to feed continuously, at least on succulent growth, so there is reason to believe that the quantity of honeydew excreted over a period of time is large. To find the exact amount would be a tedious procedure, but a relative amount was determined by counting the number of drops expelled by the aphid and measuring their diameter.

This was done with aphids from a crowded population and with those from an uncrowded population. Five groups of about 20 aphids each were taken from these populations and immediately placed on fresh succulent 8-inch plants. For a check, 23 aphids were taken from an uncrowded population and placed in a cage with no feed. The aphids from the uncrowded population (table 8) produced more honeydew than those taken from the crowded population, while aphids which were not allowed to feed on plants during the 24 hours produced no honeydew.

TABLE 8.—*Number and size of drops of honeydew excreted by pea aphids from a crowded population and by those from an uncrowded population*

Adults taken from—	Adults per plant	Drops of honeydew per 24-hour period			Adults taken from—	Adults per plant	Drops of honeydew per 24-hour period		
		Total	Average number per adult	Size			Total	Average number per adult	Size
	Number	*Number*	*Number*	*Microns*		*Number*	*Number*	*Number*	*Microns*
Uncrowded plants	20	190	9.5	600–800	Crowded plants	18	162	9.0	400–650
	20	176	8.8			19	115	6.1	
	16	166	10.4			17	146	8.6	
	18	203	11.3			15	116	7.7	
	16	157	9.8			15	125	8.3	
Total	90	892	9.9		Total	84	664	7.9	

The residues of the honeydews of the two groups of aphids appeared quite different under the microscope. This suggests that the composition varied, but as this information was obtained in only a preliminary way, no analyses were made. These observations, however, show that a careful study of this subject would add a great deal to the knowledge of the physiology of these insects.

After the completion of this investigation, a paper by Smith (*28*) appeared which describes a method of determining the relative rate of feeding of the mealy plum aphid by counting the drops of excrement thrown by the aphids onto sheets of paper. Smith found that aphids feeding as a group produced fewer drops of excrement per adult

than aphids which were isolated, and that under high temperatures fewer drops were produced than under lower temperatures.

The results of the two investigations are quite similar. Although the mealy plum aphid produced a greater number of drops per hour than the pea aphid, in both instances the aphids under the crowded condition produced fewer drops of honeydew than those under the uncrowded condition.

Since no honeydew was produced by the starved aphids, there appears to be a relationship between the volume of honeydew excreted and the intake of plant sap. The results obtained by Smith when aphids were subjected to high temperature, however, may be due not to the lack of intake of food but to the rapid evaporation of moisture from the bodies of the aphids. This investigation shows that rapid evaporation of moisture from aphids does take place, and that under low atmospheric humidity evaporation takes place more rapidly. Therefore it is not surprising that more alate forms should appear under these conditions (*23*).

ASSOCIATION OF WING APPEARANCE WITH A DECREASED VOLUME OF HONEYDEW AND A CONCENTRATION OF BODY CONTENTS

Crowding and starvation have been shown to be associated with an increase in the appearance of winged forms, and the volume of honeydew produced by aphids has been shown to decrease under such conditions. The appearance of winged forms, therefore, accompanies a decrease in the volume of honeydew excreted.

A consideration of the cause of the decreased amount of excretion brings us now to the realization that the concentration of the body content itself is associated with the appearance of wings. Evidence pointing to this was first presented in the starvation experiments, where it was shown that aphids lost weight very rapidly. After 12 hours, aphids previously weighing 2.29 mg had dropped to 1.96 mg. After 24 hours those weighing 2.41 mg had dropped to 1.88 mg (table 5). Later, in the trial moisture determination, this loss in weight was indicated as being due to loss of moisture. Aphids kept 3 to 4 hours showed a very definite decrease in moisture content as compared with those examined immediately after removal from the plants. On cloudy days this decrease was less noticeable than on bright, sunny days.

Since there were too few aphids in the starvation experiment to warrant quantitative determination, a large number of adults were collected from a pea field, mixed thoroughly in a screen cage, and then divided into three groups. Analysis of the first group was started 2 hours after collecting, that of the second group 30 hours after collecting, and that of the third group, 55 hours after collecting.

This experiment definitely showed (table 9) that as the moisture content decreased, the protein and ether extract increased. These results, which are to be expected, show more precisely the condition in which the evaporation of moisture leaves the aphid. They emphasize further that aphids not only excrete moisture in honeydew, but also give it up very rapidly by evaporation.

TABLE 9.—*Decrease in moisture content and weight, and consequent increase in protein and ether extract content of three groups of starved pea aphids*

Item	Results after—		
	2 hours	30 hours	55 hours
Weight of aphids.....grams	2.4530	1.2929	0.7291
Moisture content.....percent	72.9	65.3	50.4
Protein content.....do	12.5	19.1	30.0
Ether extract.....do	7.2	8.4	11.7

Winged appearance, therefore, is associated with concentration of body fluids brought about not only by the probable intake of more concentrated food but also by the rapid evaporation of moisture from the body of the aphid.

DISCUSSION

In the interpretation of these results, we must consider that the concentration of body fluids in the parent occurred either just before or within the period of the time of determination of the development of wings in the offspring. The length of this period is not definitely known (*25*), but whatever it is, the accumulation of the products or the concentration of body contents in the offspring must begin before this period is ended. If it occurs too early in the parent, for instance, when the prospective adults are still in the fourth stage, and if they are left on scant food, these products would be used before the adult had attained the reproductive stage, and, as was found to be the case, the progeny would have no wings. If parents are continued under unfavorable conditions, as was shown, the accumulated products would be used for the production of wings in the first young, but would not be sufficient to supply the later young. If adults are starved, as was shown, and then replaced on good succulent growth, the accumulated products would be used in the production of wings of the first young, but as the rate of the incoming fluid returned to normal and there was again enough to carry the waste products from the body of the parent, no more winged offspring would develop. Likewise, if prospective adults were kept on good succulent growth the products would not have an opportunity to accumulate and winged offspring would not develop. More winged offspring occurred on young plant seedlings, probably because the sap in these plants is more concentrated. Parents not ingesting a sufficient volume of fluid from these plants would soon have a large concentration of wing-producing products.

Winged adults subjected to unfavorable conditions would not be expected to produce many alate young because most of the products would be used in the development of their own muscles and wings. In nature they migrate to succulent growth and there the succulent food inhibits the development of wings. Only under the severest conditions would alate mothers be expected to accumulate enough of these products for the development of winged offspring.

Information presented in this investigation is applicable to circumstances wherein prediction of migrations is desired. Lack of available moisture or heavy early infestations are almost certain to

be followed by the appearance of large numbers of migrants regardless of other conditions. During the spring of 1936 a limited rainfall brought on such conditions and in one field under observation it was estimated that 90 percent of the aphids of the first generation were winged migrants. These early migrants cause an early initial infestation of field peas, which later leads to heavy losses where adequate control measures are not employed.

SUMMARY AND CONCLUSIONS

The appearance of winged offspring of the pea aphid is associated with a concentration of the body contents of the parent. The alimentary tract of the pea aphid is so constructed that it adequately eliminates waste substances only so long as fluid is ingested in sufficient volume. There appears to be no provision for voiding unnecessary products when the supply of fluid is insufficient for this purpose. Lack of fluid, together with rapid evaporation from the body of the parent aphid, causes a concentration of the waste substances. These accumulated "wastes" in the adult in the form of proteins and carbohydrates appear to initiate wing development. The appearance of apterous individuals appears to be due to (1) the lack of these "waste" substances in the parent, or (2) to the presence of a sufficient volume of fluid intake to carry them away in solution as honeydew.

LITERATURE CITED

(1) ACKERMAN, LLOYD.
1926. THE PHYSIOLOGICAL BASIS OF WING PRODUCTION IN THE GRAIN APHID. Jour. Expt. Zool. 44: 1–61, illus.

(2) BÖRNER, C.
1914. BLATTLÄUSSTUDIEN. Naturw. Ver. Bremen, Abhandl. 23: 164–184, illus.

(3) DAVIDSON, J.
1913. THE STRUCTURE AND BIOLOGY OF SCHIZONEURA LANIGERA, HAUSMANN, OR WOOLLY APHIS OF THE APPLE TREE. PART I.—THE APTEROUS VIVIPAROUS FEMALE. Quart. Jour. Micros. Soc. 58: 653–701, illus.

(4) ———
1921. BIOLOGICAL STUDIES OF APHIS RUMICIS, LINN. A.—APPEARANCE OF WINGED FORMS. B.—APPEARANCE OF SEXUAL FORMS. Roy. Dublin Soc. Sci. Proc. 16: 304–322, illus.

(5) ———
1923. BIOLOGICAL STUDIES OF APHIS RUMICIS LINN. THE PENETRATION OF PLANT TISSUES AND THE SOURCE OF THE FOOD SUPPLY OF APHIDS. Ann. Appl. Biol. 10: 35–54, illus.

(6) DAVIES, W. MALDWYN, and WHITEHEAD, T.
1935. STUDIES ON APHIDES INFESTING THE POTATO CROP. IV. NOTES ON THE MIGRATION AND CONDITION OF ALATE MYZUS PERSICAE SULZ. Ann. Appl. Biol. 22: 549–556.

(7) ELSER, E.
1924–25. BEITRÄGE ZUR QUANTITATIVEN HONIGUNTERSUCHUNG. Arch. Bienenk. 6: 118–146. (Heft 2–4, pp. [70]–98).

(8) GENTRY, THOMAS G.
1873. OBSERVATIONS ON CERTAIN PLANT LICE OF THE GENUS APHIS. Canad. Ent. 5: [121]–123.

(9) GÖLDI, EMIL A.
1885. APHORISMEN, NEUE RESULTATE UND CONJECTUREN ZUR FRAGE NACH DEN FORTPFLANZUNGSVERHÄLTNISSEN DER PHYTOPHTHIREN ENTHALTEND. 8 pp. Schaffhausen.

(10) GREGORY, L. H.
1917. THE EFFECT OF STARVATION ON WING DEVELOPMENT OF MICROSIPHUM DESTRUCTOR. Biol. Bull. 33: 296–303.

(11) HORSFALL, J. L.
1923. THE EFFECTS OF FEEDING PUNCTURES OF APHIDS ON CERTAIN PLANT TISSUES. Pa. Agr. Expt. Sta. Bull. 182, 22 pp., illus.

(12) KJELDAHL, J.
1883. NEUE METHODE ZUR BESTIMMUNG DES STICKSTOFFS IN ORGANISCHEN KÖRPERN. Ztschr. Analyt. Chem. 22: 366–382.

(13) KLODNITSKI, I.
1912. BEITRÄGE ZUR KENNTNIS DER GENERATIONSWECKSELS BEI EINIGEN APHIDIDAE. Zool. Jahrb., Abt. System., Geogr. u. Biol. Tiere 33: [445]–520, illus.

(14) KNOWLTON, GEORGE F.
1925. THE DIGESTIVE TRACT OF LONGISTIGMA CARYAE (HARRIS). Ohio Jour. Sci. 25: 244–252, illus.

(15) KYBER, JOHANN FRIEDRICH.
1815. EINIGE ERFAHRUNGEN UND BEMERKUNGEN ÜBER BLATTLÄUSE. Germar's Mag. Ent. Jahrg. 1, heft 2, pp. 1–39.

(16) MARCOVITCH, S.
1923. PLANT LICE AND LIGHT EXPOSURE. Science 58: 537–538.

(17) MASON, ARTHUR C.
1922. RELATION OF ENVIRONMENTAL FACTORS TO WING DEVELOPMENT IN APHIDS. Fla. Ent. 6: 25–32.

(18) MORDWILKO, A.
1896. ZUR BIOLOGIE DER BLATTLÄUSE AUS DEM UNTERFAMILIEN APHIDIDÆ UND PEMPHIGIDÆ. Arb. Zool. Lab. Univ. Warschau 1896: 23–146. [Title as given in Zool. Rec. 33: Ins. 45. 1896.]

(19) ———
1908. BEITRÄGE ZUR BIOLOGIE DER PFLANZENLÄUSE, APHIDIDAE PASSERRINI. Biol. Centbl. 28: 631–639, 649–662.

(20) MORGAN, ALBERT C. F.
1885. NOTES ON EXPERIMENTS MADE WITH THE WINGED FORM OF PHYLLOXERA VASTATRIX RADICICOLA. Ent. Soc. London, Trans. 1885: xxvii–xxxii.

(21) MORGAN, T. H.
1909. A BIOLOGICAL AND CYTOLOGICAL STUDY OF SEX DETERMINATION IN PHYLLOXERANS AND APHIDS. Jour. Expt. Zool. 7: [239]–352, illus.

(22) REINHARD, H. J.
1927. THE INFLUENCE OF PARENTAGE, NUTRITION, TEMPERATURE, AND CROWDING ON WING PRODUCTION IN APHIS GOSSYPII GLOVER. Tex. Agr. Expt. Sta. Bull. 353, 19 pp.

(23) RIVNAY, E.
1937. MOISTURE AS THE FACTOR AFFECTING WING DEVELOPMENT IN CITRUS APHIS, TOXOPTERA AURANTII, BOY. Bull. Ent. Research 28: 173–179.

(24) SHULL, A. FRANKLIN.
1926. LIVE-CYCLE IN APHIDS AFFECTED BY DURATION OF LIGHT. (Abstract) Anat. Rec. 34: 168–169.

(25) ———
1929. DETERMINATION OF TYPES OF INDIVIDUALS IN APHIDS, ROTIFERS AND CLADOCERAE. Cambridge Phil. Soc. Biol. Rev. and Proc. 4: 218–248.

(26) SLINGERLAND, M. V.
1893. NOTES FROM THE CORNELL INSECTARY. II.—SOME OBSERVATIONS UPON PLANT-LICE. Science 21: 48–49.

(27) SMITH, KENNETH M.
1926. A COMPARATIVE STUDY OF THE FEEDING METHODS OF CERTAIN HEMIPTERA AND OF THE RESULTING EFFECTS UPON THE PLANT TISSUE WITH SPECIAL REFERENCE TO THE POTATO PLANT. Ann. Appl. Biol. 13: 109–139, illus.

(28) SMITH, LESLIE M.
1937. GROWTH, REPRODUCTION, FEEDING, AND WING DEVELOPMENT OF THE MEALY PLUM APHID IN RELATION TO CLIMATIC FACTORS. Jour. Agr. Research 54: 345–364, illus.

(29) TANNREUTHER, GEO. W.
1907. HISTORY OF THE GERM CELLS AND EARLY EMBRYOLOGY OF CERTAIN APHIDS. Zool. Jahrb., Abt. Anat. u. Ontog. Tiere 24: [609]–642, illus.

(30) UMBREIT, W. W., and BOND, V. S.
1936. ANALYSIS OF PLANT TISSUE. APPLICATION OF A SEMIMICRO-KJELDAHL METHOD. Indus. and Engin. Chem., Analyt. Ed. 8: 276–278.
(31) WADLEY, F. M.
1923. FACTORS AFFECTING THE PROPORTION OF ALATE AND APTEROUS FORMS OF APHIDS. Ann. Ent. Soc. Amer. 16: 279–303, illus.
(32) WALKER, FRANCIS.
1873. NOTES ON APHIDES. Canad. Ent. 5: 172–173.
(33) WARREN, ERNEST.
1902. VARIATION AND INHERITANCE IN THE PARTHENOGENETIC GENERATIONS OF THE APHIS HYALOPTERUS TRIRHODUS (WALKER). Biometrika 1: [129]–154, illus.
(34) WIGGLESWORTH, V. B.
1934. INSECT PHYSIOLOGY. 134 pp., illus. London.
(35) WOODWORTH, C. W.
1908. WINGED APHIDS. Ent. News 19: 122–123.

THE GROWTH CURVE OF SORGHUM[1]

By A. T. BARTEL, *assistant agronomist*, and J. H. MARTIN, *senior agronomist*, *Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*[2]

INTRODUCTION

Growers and investigators of sorghum (*Sorghum vulgare* Pers.) generally have observed the small size of seedlings and the relatively slow early growth of the plants, as compared with those of corn, even when grown under optimum conditions. Measurements of the growth of sorghum plants at some stages doubtless have been made previously, but curves showing the trend of this slow early growth have not been found in the literature. In 1930, N. V. Kanitkar, then soil physicist of the Poona Agricultural College, Poona, India, while visiting the United States, showed the junior writer an unpublished growth curve of sorghum based upon data he had obtained in India. This curve was of the unbalanced sigmoid type, having a long gradual slope during the first several weeks of the period, that depicted strikingly the slow early growth and rapid later growth of sorghums. It seemed desirable to the writers to investigate this problem in the United States and if possible to determine the cause of this unbalanced trend in growth rate. The experiments were conducted by the senior writer.

Sorghums are of tropical origin and usually grow slowly at the cool temperatures that frequently occur after relatively early planting in the Northern States. The sorghums in the experiments reported here, however, were planted in June and July under conditions of high temperature and irrigation in southern Arizona, which favored rapid germination and early growth. Thus unfavorable environmental conditions that might have retarded early growth were largely eliminated from consideration as a causal factor in the growth trend.

METHODS

The experiments were conducted at the University Farm, Tucson, Ariz., under irrigation, during the 4-year period 1931–34 and in 1936. Two grain sorghum varieties, Dwarf hegari and Double Dwarf Yellow milo, were planted on two dates in each of the first 4 years. The average date for the first planting was June 18 and for the second planting July 22. Soon after the plants were well up they were thinned to a single plant every 8 to 10 inches. The plants of both varieties produced tillers that developed about as well as the main stalks. Plants were harvested at 4-day intervals starting soon after the plants were up and ending when maturity was reached. The plants were cut as close to the ground as possible and a minimum of 10 plants was harvested at each sampling. The number of stalks (in-

[1] Received for publication April 8, 1938; issued December 1938. Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Arizona Agricultural Experiment Station.

[2] The authors acknowledge the assistance of Charles Davis, assistant agronomist, Arizona Agricultural Experiment Station, in calculating the growth curves.

cluding tiller stalks) was counted in each sample, and all weights were computed to weight per stalk. The harvested plants were weighed at intervals until they had reached a constant air-dry weight. The heads, if any, were then removed and threshed, and weights of the heads and seed were obtained.

RESULTS

RATE OF GROWTH IN SORGHUMS

The increase in weight per stalk, including the heads, seeds, and leaves, of Dwarf hegari and Double Dwarf Yellow milo planted on two dates is shown in figure 1. The curves were calculated by the formula suggested by Robertson,[3] i. e., $\text{Log}\frac{x}{a-x}=K(t-t_1)$, where x equals the weight attained in time t; a is the total growth attained during the cycle; K is a constant, the magnitude of which determines the slope of the curve; and t_1 is the time at which the growth is one-half completed; or where x equals $1/2a$. It will be observed that the early growth of both varieties was very slow. This was especially true in the June 18 planting. It was not until 54 days after planting that a weight per stalk of over 10 g was obtained in Dwarf hegari. This period constitutes roughly one-half of the number of days between planting and maturity. The early growth of Double Dwarf Yellow milo was slightly greater than that of Dwarf hegari until about 58 days after planting. From then until maturity the weight per stalk of Dwarf hegari increased more rapidly than in the shorter variety Double Dwarf Yellow milo. The weight per stalk 54 days after planting represented 10 percent of the weight at maturity of Dwarf hegari and 14 percent of Double Dwarf Yellow milo.

A theoretical[4] symmetrical sigmoid (S-shaped) curve of growth, typical of many plant species, also is shown in figure 1. This curve is superimposed upon the weights of the Dwarf hegari planted on June 18. It will be observed that the curve of actual dry-weight increase of Dwarf hegari was below that of the theoretical curve at all stages of development until maturity was reached. At maturity, of course, the two curves coincide.

The growth curves of sorghum show a delayed but more abrupt upward trend, with the upper and lower ends asymmetrical in contrast to the symmetrical theoretical curve. The difference may be explained by the small size of the sorghum seedling relative to that of the mature plant, which necessitates the lapse of considerable time before the operation of the so-called "compound-interest" principle can produce large increases in weight.

When the two varieties were planted on July 22 there was a more rapid increase in early growth and a lower weight at maturity than when they were planted on June 18. This rapid early growth might be attributed to slightly higher temperatures at planting time in the July 22 planting. The mean temperatures at Tucson for June, July, and August from 1931 to 1934 were 79.5°, 87.6°, and 84.4° F., respectively. When the varieties were planted on June 18 maturity was reached the first week in October, but when planted on July 22

[3] ROBERTSON, T. BRAILSFORD. THE CHEMICAL BASIS OF GROWTH AND SENESCENCE. 389 pp., illus. Philadelphia and London. 1923.

[4] RABER, ORAN. PRINCIPLES OF PLANT PHYSIOLOGY. 377 pp., illus. New York. 1928.

the varieties were not mature until the last week in October. The lower mean temperatures and shorter days during part of the growing period of the July plantings may have hastened heading and restricted

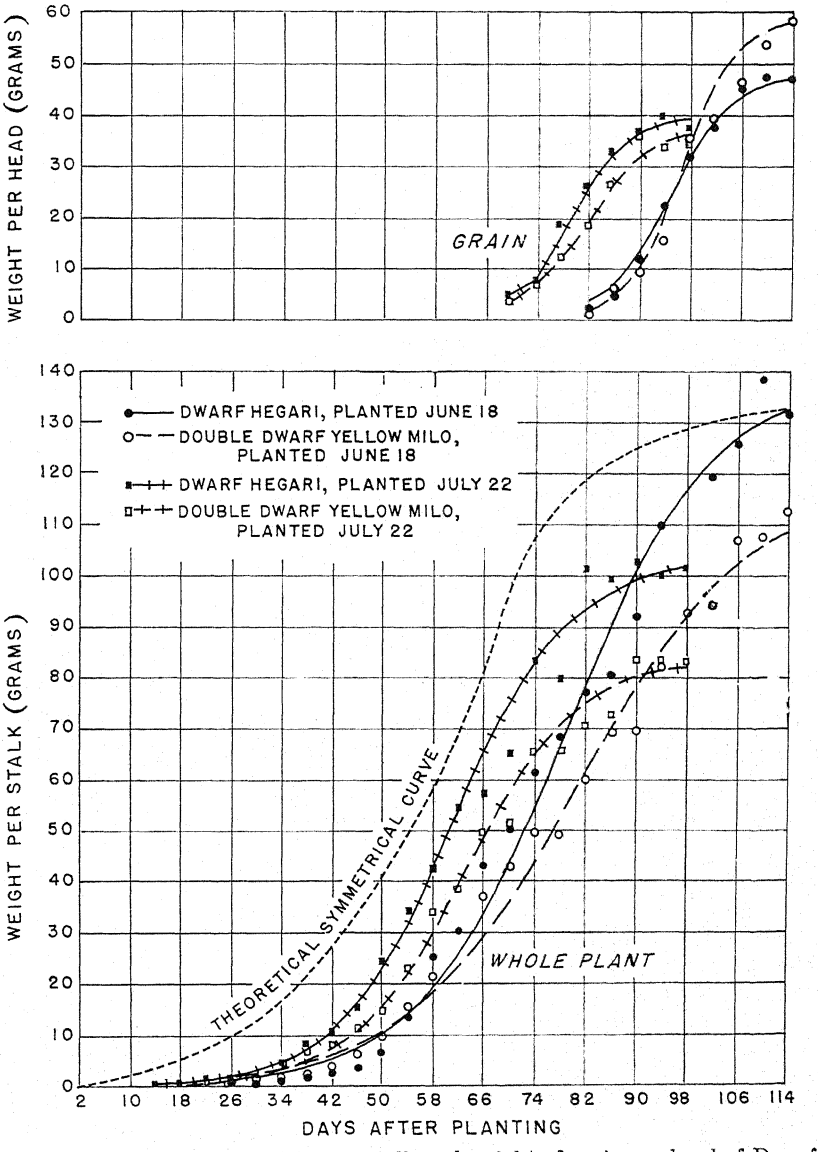


FIGURE 1.—Total air-dry weight per stalk and weight of grain per head of Dwarf hegari and Double Dwarf Yellow milo planted on two dates at Tucson, Ariz., 1931–34. The dots and circles represent the actual weights, and the lines the fitted curves.

vegetative growth, although the temperatures were sufficiently high during October to prevent delay in ripening.

The increase in seed weight per stalk of Dwarf hegari and Double Dwarf Yellow milo in the June 18 and July 22 plantings also is shown in figure 1. The poor fit in the curve for seed weight of Double Dwarf Yellow milo planted in June is due to damage by birds. However, both varieties yielded more in the June than in the July planting. In the June planting the seed yield of Double Dwarf Yellow milo was higher, but Dwarf hegari led in the July planting. The decreases as a result of later planting in weight per stalk and seed weight per head were 23 percent and 19 percent, respectively, in Dwarf hegari, and 26 percent and 41 percent in Double Dwarf Yellow milo. The Dwarf hegari evidently was better suited to late planting than Double Dwarf Yellow milo.

SEED SIZE AND EARLY GROWTH

In numerous experiments with sorghum seedlings, the junior writer has observed that varieties with large seeds produce larger seedlings and appear to grow more rapidly in the early stages than small-seeded varieties. It seemed that this apparent relationship between the size of seed and early plant growth might explain the growth trends just presented.

Mature stalks of some sorghum varieties often weigh nearly as much as mature cornstalks, although the seeds of grain sorghums are much smaller and usually weigh only about as much as average caryopses of wheat, oats, and barley; and seeds of some of the sorgos, particularly Sumac, weigh still less. Proso (*Panicum miliaceum* L.), a grain millet, has extremely small seeds for a cereal crop and makes a correspondingly slow early growth. The sorghums and proso in general have a wider ratio between seed size and final stalk weight at maturity than any other of the American cereal crop plants.

Plantings of cereal varieties having different seed sizes were made on July 13 and August 11, 1936. Included in this experiment were two varieties of corn (*Zea mays* L.), Surecropper and Krug; four varieties of sorghum (feterita, Double Dwarf Yellow milo, Dwarf hegari, and Sumac sorgo); and one variety of proso (Yellow Manitoba). The oven-dry weights per kernel, and per stalk 10 days after planting, of each variety are shown in table 1, and the stalk weights at intervals during periods up to 40 days after planting, which include only a part of the grand period of growth, are shown in figure 2. The stalk weights shown are averages from the two plantings.

In general, a close relationship was found between the size of seed and the size of seedling. The corn variety Surecropper had the largest seed and also the most rapid early growth, while Yellow Manitoba proso had the smallest seed and the least early growth. Krug corn made less early growth than the larger-seeded Surecropper variety but exceeded all of the sorghums. Among the sorghum varieties, feterita had the largest seeds and grew most rapidly during early stages, while Sumac sorgo had the smallest seeds and slowest apparent early growth. A distinct spread between sorghum varieties occurred from the sixteenth to the twentieth day after planting and the stalk weights then were in the same order as the seed weights, except that Dwarf hegari and Double Dwarf Yellow milo were reversed. The seeds of the latter variety usually are larger than those of Dwarf hegari but in these samples they were slightly smaller. The early growth of Double Dwarf Yellow milo exceeded that of Dwarf hegari, as shown in figure 1.

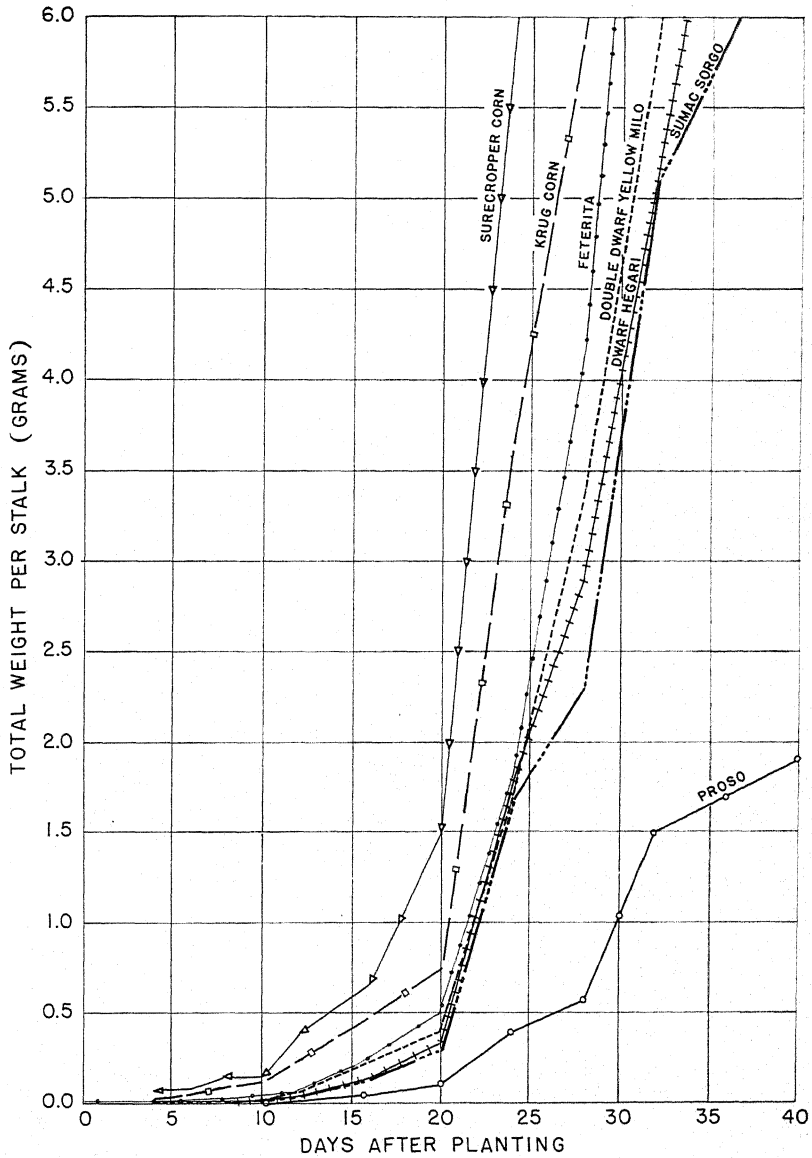


FIGURE 2.—Oven-dry weight per stalk of two varieties of corn, four of sorghum, and one of proso, at Tucson, Ariz., in 1936.

TABLE 1.—*Oven-dry weight per seed, and per stalk 10 days after planting and at different periods thereafter, of 7 cereal varieties*

Crop and variety	Weight per seed	Weight per stalk 10 days after planting	Period after planting for weight per stalk to equal weight per seed
	Mg	*Mg*	*Days*
Corn:			
Surecropper	407.8	149	14
Krug	188.0	112	11
Sorghum:			
Feterita	26.9	38	9
Dwarf hegari	25.9	33	9
Double Dwarf Yellow milo	23.4	37	8
Sumac sorgo	14.2	20	8
Proso:			
Yellow Manitoba	3.1	12	5

An inspection of figure 2 shows that after the seedlings had reached a weight of roughly 0.5g per stalk, the growth-curve rise for the next 5 days was strikingly and almost uniformly abrupt in all varieties. This is in conformity with the monomolecular autocatalytic law, upon which the usual growth curve is based,[5] in which increase in weight varies with the "active mass."

The weight per stalk 10 days after planting, which was previous to a pronounced upturn in the growth curve of any of the cereal varieties, is compared graphically with the weight per seed in figure 3, *A*. A

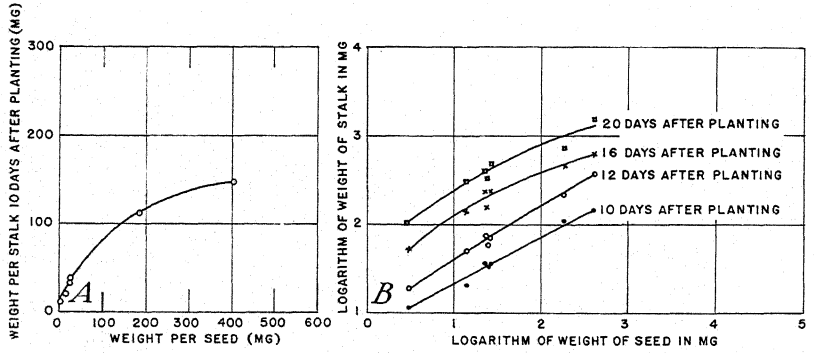


FIGURE 3.—*A*, Relation between weight per seed and weight per stalk 10 days after planting of corn, sorghum, and proso; *B*, relation between logarithms of the seed weights and of the stalk weights taken at various intervals up to 20 days after planting.

curvilinear relationship between stalk weight and seed size is indicated that closely approaches a logarithmic curve. When the logarithms of the two sets of weights are charted (fig. 3, *B*) the points fall approximately on a straight line. The relationship was linear also 12 days after planting. Later, 16 and 20 days after planting, when appreciable growth was occurring, the corresponding points in figure 3, *B*, form slight curves instead of straight lines, which indicates that factors other than seed size were operative in partly determining the weight per stalk at these stages. Ten and twelve days after

[5] ROBERTSON, T. BRAILSFORD. See footnote 3.

planting the logarithm of the weight of the seedling stalks thus varied directly with the logarithm of the weight of the seed of the corn, sorghum, and proso varieties included in the experiment. This is in conformity with the calculation of growth curves, which is based upon a logarithmic relationship between the weight of the plant (or "active mass") and the increase in growth. The results strongly suggest that the size of 10-day-old seedlings of the cereals investigated was determined by the mass of the seed and the apparent slow early growth of the sorghums and proso was merely a logarithmic function of seed size. A growth curve for barley obtained by Pope [6] showed a trend in early growth very similar to that of the sorghums shown in figure 1. Caryopses of barley have approximately the same average weight as those of the grain sorghums Dwarf hegari and Double Dwarf Yellow milo.

Ashby [7] has suggested that vigor in corn hybrids may be due in part to larger embryos, although this view has been challenged by Sprague [8] and others. Incidentally, Sprague's growth data, with one exception, show that the logarithms of seed and seedling weights are proportional.

SUMMARY

Sorghum seedlings are smaller and appear to be slower in early growth than those of corn. The increases in dry weight per stalk and grain weight per head of two grain sorghum varieties, Dwarf hegari and Double Dwarf Yellow milo, grown in the field under irrigation at Tucson, Ariz., from 1931 to 1934, are presented. A planting at a normal date, about June 18, and a later planting, about July 22, were made in each of the 4 years.

About 10 to 15 percent of the final dry weight of the stalks and heads was produced during the first half of the growing period. The growth curves of the two varieties in both plantings indicated a much slower increase in growth in early stages of development and a more rapid increase at later stages than would be expected from the usual symmetrical sigmoid growth curve typical of many plants.

Two varieties of corn, four of sorghum, and one of proso, representing a wide range of seed sizes, were planted in the field in 1936, and the rate of early growth was determined. A close relationship was found between the size of seed planted and the dry weight per stalk in the early stages of plant development. In young seedlings of corn, sorghum, and proso, 10 and 12 days after planting, the logarithm of the weight per stalk was directly proportional to the logarithm of weight per seed. Seedling size thus was a logarithmic function of the "active mass" in the seed. The wider ratio between the weight of the seed and the weight of the mature stalk appears to explain for the most part the smaller seedlings and apparent slower early growth of sorghum and proso, as compared with corn, under optimum growing conditions.

[6] POPE, MERRITT N. THE GROWTH CURVE IN BARLEY. Jour. Agr. Research 44: 323-341, illus. 1932.

[7] ASHBY, ERIC. STUDIES IN THE INHERITANCE OF PHYSIOLOGICAL CHARACTERS. 1. A PHYSIOLOGICAL INVESTIGATION OF THE NATURE OF HYBRID VIGOUR IN MAIZE. Ann. Bot. [London] 44: [457]-467, illus. 1930.

[8] SPRAGUE, G. F. HYBRID VIGOR AND GROWTH RATES IN A MAIZE CROSS AND ITS RECIPROCAL. Jour. Agr. Research. 53: 819-830, illus. 1936.

CORRELATIONS BETWEEN BIOLOGICAL ESSENTIALITY AND ATOMIC STRUCTURE OF THE CHEMICAL ELEMENTS [1]

By ROBERT A. STEINBERG [2]

Associate physiologist, Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

An examination of the data on the mineral nutrition of organisms led to the discovery of close correlations between the list of the known essential elements and the quantitative data of atomic physics, permitting of tentative, if vague, deductions as to their total number and the identity of those as yet unknown. Though these correlations have been evident to the writer for some time, considerable hesitancy has been felt in calling attention to them in view of the importance of the questions involved and the possibility of error in interpretation of the data. The positive proof subsequently available of the essentiality of manganese and the discovery of the essentiality of molybdenum (*1*, *6*),[3] cobalt (*9*), and gallium (*7*), which one after another have fallen into their proper niches in the tables bringing out these relations, at length convinced the writer that these data should be made available for critical examination by other investigators. Substantiation would imply that the particular chemical elements required in the functioning of protoplasm are essential because of the inner physics of the atoms, and the quantum-mechanic relations of the atoms of these elements to one another.

REVIEW OF LITERATURE

Casual comments on the elements required by organisms are numerous and range from the assumption that organisms may require all 92 elements to one that some organisms have special requirements not shared by others. Attempts at correlations have been few and not very successful, owing to the limited knowledge hitherto available. Thatcher (*8*) proposed a classification of the chemical elements based on their biological properties, whereas Frey-Wyssling (*2*) considered that he had found a relation to exist between biological essentiality and position in the chemical periodic table. Lendle (*3*), on the other hand, confined himself to pointing out analogies in biological effects of homologous elements. Pirschle (*4*, *5*) has reported at length on the effects of homologous elements in *Aspergillus*.

RELATION OF BIOLOGICAL ESSENTIALITY TO ATOMIC STRUCTURE

A comparison of the essential [4] with the nonessential elements of the standard chemical periodic table with respect to position discloses evidence of considerable interest. This is brought out in table 1,

[1] Received for publication August 8, 1938; issued December, 1938.

[2] The writer acknowledges his indebtedness to Dr. F. L. Mohler, Chief of the Section of Atomic Physics, Bureau of Standards, U. S. Department of Commerce, for aid in the preparation of this manuscript.

[3] Italic numbers in parentheses refer to Literature Cited, p. 851.

[4] All the chemical elements definitely known to be essential, whether for animals or plants, are included. Limitations of space prevent the inclusion of full citations, nor are they nesessary in the case of the majority of the elements.

a reproduction of the standard chemical periodic table in condensed form, showing only the positions of the essential elements. Several facts stand out. Three, and no more than three, of the essential elements are found in each of five of the nine groups. Three contain two elements each. Each group containing three essential elements has one or two of them in the main group and not more than one in one or both of the subgroups. Groups 0 and VIII appear to form exceptions to these statements. Hydrogen, however, may be assumed to be a member of group 0, in which there are, of course, no subgroups. Group VIII contains two essential elements, but perhaps provision should be made for the addition of a third for reasons that will appear later. It may be noted that hydrogen and the two groups requiring special mention with respect to the property of essentiality are the same that have caused difficulty to the chemist in the classification of the chemical elements. On the basis of these facts, it seems probable that a total of three essential elements will also be found eventually in groups IV and V. The difficulties of table 1 are inherent in the chemical periodic table as a medium for bringing out these biological correlations.

TABLE 1.—*The biologically essential elements arranged in the form of the chemical periodic table*

Elements in the chemical periodic table in group [1]—

0	I		II		III		IV		V		VI		VII		VIII	
H	Na		Mg		B		C Si		N P		O S		Cl			
	K	Cu	Ca	Zn	(Sc)[2]	Ga	[3]*	*	*	*	Mo		Mn	I	Fe	Co

[1] Group 0 may be considered to contain no subgroups and group VIII no main group.
[2] Other evidence on hand renders quite probable the essentiality of scandium to *Aspergillus niger*, but will be presented at another time in connection with other matters if and when its essentiality to this organism is further verified.
[3] An asterisk (*) represents both nonessential elements and elements whose biological essentiality as yet remains unproved.

Additional correlations between biological essentiality and chemical periodicity are evident if the essential elements are arranged in a series of groups (table 2). When arranged in a series according to atomic number the essential elements form groups, of one to four members, which appear to bear definite numerical relations to each other. This is made plain by changes in the number of members of consecutive groups. The discrepancies in the number of group members of the elements having higher atomic numbers are due in all probability to blanks caused by the omission of essential elements not yet identified. The differences between the atomic numbers of the first members of these series groups also exhibit definite regularities, though here again unavoidable omissions cause irregularities in the values between groups of higher atomic number. Similarly, the numbers of elements omitted between consecutive groups of known essential elements are 3, 2, 1, 1, 3, 1, 10, and 10, respectively. Silicon has been included as an essential element because of its use for cell-wall construction by the Diatomaceae, though its essentiality has never been demonstrated and its omission increases the regularities

initial element of each group has an odd atomic number. These are silicon (atomic number 14) and molybdenum (atomic number 42). It is not impossible, therefore, that silicon may eventually be determined to be nonessential and columbium essential. Another interesting feature of series-group arrangement is the differences in atomic number of the initial members of the positive-element groups. These differences are 10, 8, 6, 4, and 13 for consecutive groups. The difficulties with table 2 as a means of bringing out correlations are inherent in the use of series groups.

TABLE 2.—*The group periodicity of biologically essential elements as a function of atomic number*

Group No.	Symbols of elements, preceded by atomic numbers [1]	Members in indicated group	Difference between atomic numbers of first members of consecutive groups	Nonessential elements between consecutive groups of known essential elements
		Number		*Number*
1	(1,H)	1	------	--------
2	(5, B)-(6, C)-(7, N)-(8, O)	4	4	3
3	(11,Na)-(12,Mg)	2	6	2
4	(14,Si)-(15,P)-(16,S)-(17, Cl)	4 [2](3)	3 [2](4)	1 [2](2)
5	(19,K)-(20,Ca)-(21, (Sc))	3	5 [2](4)	1
6	(25,Mn)-(26,Fe)-(27,Co)	3	6	3
7	(29,Cu)-(30,Zn)-(31,Ga)	3	4	1
8	(42,Mo)	1	13	10
9	[3]*	?	?	?
10	(53,I)	1	11	10

[1] Atomic numbers in each group are consecutive.
[2] Value if silicon is considered unessential.
[3] The asterisk (*) represents a group of elements whose biological essentiality as yet remains unproved.

Correlations between essentiality and the inner physics of the atom can be shown best, however, through the tabulation of the 92 elements on the basis of their transition subshells, or the subshells in which the electron numbers of the atoms have undergone a *regular* change in the formation of the elements. These are the electrons that determine to a large extent the optical and chemical properties of the elements of which they form a part. Table 3 presents the chemical elements arranged, on the basis of transition subshells, according to shell, subshell, atomic number, and "rank," to bring out the relation of atomic structure to the property of biological essentiality. The atomic data for the individual elements in the normal state are those given by White (*10*). The shells into which the electrons are arranged about the nucleus of the atom are designated as n=1, n=2, n=3, n=4, n=5, n=6, and n=7 from the nucleus outward. Each shell may comprise one or more subshells, designated as l=0, l=1, l=2, and l=3, also numbered from the center. The symbol for each element is preceded by the atomic number of the element. The numbers 1 to 14 at the heads of columns indicate the order in which the elements occur in each subshell row when arranged according to the magnitude of their atomic numbers. For these values the term "rank" will be used. This arrangement is more fundamental than that of the standard chemical periodic table inasmuch as spatial relations as well as number

TABLE 3.—*The chemical elements arranged on the basis of the subshell undergoing regular change in electron number* [a]

Shell No.	Subshell No.	Atomic numbers and symbols of chemical elements [c] in indicated rank [b]														Elements per shell		Known essential elements per shell		Elements per shell according to Rydberg	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	Number	Factors	Number	Factors	Number	Factors
n=1	l=0	*1, H*	2, He													2	2×1^2	1	1^2	2	2×1^2
n=2	l=0	3, Li	4, Be													8	2×2^2	4	2^2	8	2×2^2
	l=1	*5, B*	*6, C*	7, *N*	*8, O*	9, F	10, Ne														
n=3	l=0	*11, Na*	*12, Mg*													18	2×3^2	12	3×2^2	8	2×2^2
	l=1	13, Al	*14, Si*	*15, P*	*16, S*	*17, Cl*	18, A														
	l=2	*21, Sc*	22, Ti	23, V	24, Cr	*25, Mn*	*26, Fe*	*27, Co*	28, Ni	*29, Cu*	*30, Zn*										
n=4	l=0	*19, K*	*20, Ca*													32	2×4^2	4	2^2	18	2×3^2
	l=1	*31, Ga*	32, Ge	33, As	34, Se	35, Br	36, Kr														
	l=2	39, Y	40, Zr	41, Cb	*42, Mo*	43, Ma	44, Ru	45 Rh	46, Pd	47, Ag	48, Cd										
	l=3	58, Ce	59, Pr	60, Nd	61, Il	62, Sm	63, Eu	64, Gd	65, Tb	66, Dy	67, Ho	68, Er	99, Tm	70, Yb	71, Lu						
n=5	l=0	37, Rb	38, Sr													18	2×3^2	1	1^2	18	2×3^2
	l=1	49, In	50, Sn	51, Sb	52, Te	*53, I*	54, Xe														
	l=2	57, La	72, Hf	73, Ta	74, W	75, Re	76, Os	77, Ir	78, Pt	79, Au	80, Hg										
	l=3																				
n=6	l=0	55, Cs	56, Ba													12	3×2^2	0	0	32	2×4^2
	l=1	81, Tl	82, Pb	83, Bi	84, Po	85	86, Rn														
	l=2	89, Ac	90, Th	91, Pa	92, U																
	l=3																				
n=7	l=0	87	88, Ra													2	2×1^2	0	0	6	2×3
	l=1																				
	l=2																				
	l=3																				
Total elements per rank		17	17	10	10	9	9	4	4	4	4	1	1	1	1						
Essential elements	l=0	H, Na, K	Mg, Ca																		
	l=1	B, Ga	C, Si	N, P	O, S	Cl, I															
	l=2	(Sc)	[c] *	*	Mo	Mn	Fe	Co	*	Cu	Zn										
	l=3	*																			
Known essential elements per rank		6	4	2	3	3	1	1	0	1	1										

[a] The chemical elements are indicated by their chemical symbols and are preceded by their atomic numbers. Italics indicate essential elements.
[b] The "rank" represents the relative order of the elements of each subshell when arranged according to atomic number.
[c] Asterisks (*) indicate elements the biological essentiality of which has not been proved.

At the bottom of table 3 is what may be regarded as a summation of these data with respect to the biologically essential elements. The five essential elements in subshell l=0 are all positive, and their number decreases from 3 in rank 1 to 2 in rank 2. The 10 essential elements formed by a change in subshell l=1 are arranged uniformly in pairs in ranks 1 to 5, inclusive. Gallium occupies a position among these negative elements, whereas in table 2 it fell among the positive ones. Finally, the l=2 subshell row with seven elements appears to contain but a single essential element in each rank. The three blank spaces may represent elements the essentiality of which has not yet been proved. No element essential for nutrition occurs in the l=3 row, which includes the rare earths only, though the essentiality of cerium,[5] particularly, is not definitely eliminated. The total number of known essential elements in successive shells forms a very uniform series, namely, 1, 4, 12, 4, 1, 0, and 0, reckoned from the nucleus to the periphery of the atom. Sodium, calcium, boron, (gallium), chlorine, and iodine—forming a group of essential elements dispensed with by certain organisms—fall into terminal ranks of the subshells. The segregation of the essential elements into positive and negative groups and the progressive decrease in the number of such elements per rank in successive subshells, together with the uniformity in number of elements per rank in each subshell, cannot be attributed to chance. These regularities, rather, must be interpreted as an expression of the interplay of ordered forces having their seat in the mechanism of atomic structure.

The objection might be raised that the use of table 3, and particularly of the property termed the "rank," is unacceptable because of its wide departure from the arrangement of the Mendelejeff chemical periodic table. Nevertheless, this table, though of greater complexity, also serves to bring out the effects of position and number of electrons on the chemical and physical properties of the elements as well as or better than the standard table. Moreover, the places into which the elements fall are definite and without equivocation. Subshells l=0 and l=1 seem to be the positions particularly associated with the possession of metalloid properties, a characteristic that increases with the rank until the gaseous state is reached. Shell position, however, is also of some effect in this respect. Two of the series of elements afford a complete illustration of the Rydberg rule for increases in atomic number of homologous elements. Beginning with hydrogen, the elements of subshell l=0 in rank 1 increase in atomic number in successive amounts of 2, 8, 8, 18, 18, and 32. The same is true for the elements of subshell l=0 in rank number 2 beginning with helium. Other correlations are readily apparent. It would not be surprising, however, if certain discrepancies exist, since only three of the quanta of the atom and the rank form the basis of this classification. The characteristics of beryllium and magnesium would be expected to be less metallic. Ample chemical justification exists, nevertheless, for the use of this table to bring out the association between atomic structure and biological essentiality, and to employ it as a basis to demonstrate that the elements possessing the property of essentiality form not a random selection but a group correlated with respect to atomic structure.

[5] Cerium perhaps should occupy the position of hafnium, and the latter that after lutecium in the l=3 subshell.

As a matter of fact, the arrangement of table 3 is an improvement in certain respects over that of the standard table. Hydrogen finds a definite position, as do also the rare-earth metals and the members of group VIII. Changes in electron number in subshell l=3 effect little change in chemical properties of the elements as exemplified by the rare earths, which alone of all the elements occupy this position. The total number of elements in the shells differs from that of the Rydberg (*10*) series in being much more symmetrical. If each rank be divided into four subranks equivalent to positions in subshells l=0, l=1, l=2, and l=3, the elements arrange themselves into series quite similar to those of the familiar groups and subgroups of the standard periodic table. The relative positions of the elements are more in keeping with their chemical properties than in the standard table, however. Another difference lies in the number of ranks required to bring about a periodic change in properties of the elements. In subshell l=0, the equivalent of 2 elements, in subshell l=1, the equivalent of 6 elements, and in subshell l=2, the equivalent of 10 elements is required. That is, the periodic recurrence of properties with increase in atomic number differs uniformly with the transition subshells in the ratio of 2:6:10; or, if the rare-earth elements are included, the series is 2:6:10:14. Chemical valence is associated with the subrank and shows far fewer anomalies than in the Mendelejeff table. The number of elements per rank also forms an interesting series, namely, 17, 17, 10, 10, 9 9, 4, 4, 4, 4, 1, 1, 1, and 1. The pairing evident in this series is in accordance with the pairing shown by all the 92 elements and by the majority of the essential elements in this table. The factors for this series are 4^2+1, 3^2+1, 3^2+0, 2^2+0, and 1^2+0. Though its significance is unknown to the writer, its regularity and the uniformity of pairing in the values is believed to have a basis in atomic structure and serves to strengthen the belief that the correlations between biological essentiality and atomic structure based on the ranks of table 3 have an objective existence.

DISCUSSION

The deductions feasible on the basis of the correlations between atomic structure and essentiality with respect to the total number of essential elements and of their identity are vague, largely negative in character, and debatable. It appears probable from the data of table 1 that at least two, and perhaps three, additional elements will be found to be essential. These, it is assumed, would occur singly in groups IV and V and perhaps in group VIII. The data of table 2 are almost too ambiguous for prognostication, though the nonessentiality of silicon and the essentiality of columbium are indicated. Consideration of the data in table 3 would indicate to the writer that no more elements of shells n=1, n=2, and n=3 will be found essential; nor, if greatest weight is placed on the apparent regularity of the series of the total number of essential elements per shell, are any to be found in the other shells. However, the nonessentiality of silicon would indicate an unidentified essential element in shell n=3. The irregularities in the series formed by the total number of known essential elements per rank and the vacancies in the l=2 subshell row of essential elements may perhaps indicate otherwise. Mathematically, it would be practicable to fill the vacancies in subshell l=2 without seriously affecting

the uniformity of the series of total essential elements per shell, though an alteration would be necessary.

A thorough understanding of factors concerned should permit foreknowledge of experimental results. Nevertheless, attempted predictions in scientific investigations, as elsewhere, have been found extremely hazardous and are to be avoided as of dubious value, unless strongly justified as in this case. Search for the essential trace elements in the past has largely been purely empirical and directed almost by chance. The publication of these tables will, it is hoped, act as a beneficial directive influence, since a direct objective is now available that makes feasible the partial correlation of the data on the essential elements. It should not be forgotten, however, that no single organism seems to require all (22 or more) essential elements. This situation may be purely illusory and depend on low experimental precision, but it is probably associated with broad evolutionary trends that include gain or loss of function. The belief of the writer in the existence of only a few other as yet unidentified essential elements may therefore need considerable revision on the basis of experience with organisms other than *Aspergillus*. The writer is also convinced that eventually an even closer correlation between essentiality and atomic structure will be demonstrated by the atomic physicist through the mathematically complex quantum mechanics of the atom.

SUMMARY

Correlations were found between atomic structure and biological essentiality of the chemical elements that would indicate that the essential elements are closely correlated with respect to atomic structure and their distribution among the nonessential elements. Tentative, though vague and debatable, deductions are feasible with respect to the number and identity of the essential elements as yet unknown. A suggested form of chemical periodic table based on shell and subshell of transition, atomic number, and rank, is superior in certain respects to the standard table. Moreover, this arrangement of the chemical elements makes possible the correlation of atomic structure with the property of biological essentiality.

LITERATURE CITED

(1) Bortels, H.
1930. Molybdän als katalysator bei der biologischen stickstoffbindung. Arch. Mikrobiol. 1: [333]–342.

(2) Frey-Wyssling, A.
1935. Die unentbehrlichen elemente der pflanzenernährung. Naturwissenschaften 23: 767–769, illus.

(3) Lendle, A.
1936. Periodisches system und biologische wirkung. Chem. Ztg. 60: [833]–835.

(4) Pirschle, Karl.
1934. Vergleichende untersuchungen über die physiologische wirkung der elemente nach wachstumversuchen mit aspergillus niger (stimulation und toxizität.) Planta 23: [177]–224.

(5) ———
1935. Weitere untersuchungen über die vergleichsweise wirkung der elemente nach wachstumversuchen mit aspergillus niger (stimulation und toxizität). Planta 24: [649]–710.

(6) STEINBERG, ROBERT A.
1936. RELATION OF ACCESSORY GROWTH SUBSTANCES TO HEAVY METALS, INCLUDING MOLYBDENUM, IN THE NUTRITION OF ASPERGILLUS NIGER. Jour. Agr. Research 52: 439–448.

(7) ———
1938. THE ESSENTIALITY OF GALLIUM TO GROWTH AND REPRODUCTION OF ASPERGILLUS NIGER. Jour. Agr. Research 57:569–574.

(8) THATCHER, R. W.
1934. A PROPOSED CLASSIFICATION OF THE CHEMICAL ELEMENTS WITH RESPECT TO THEIR FUNCTIONS IN PLANT NUTRITION. Science 79: 463–466.

(9) UNDERWOOD, E. J.
1937. COBALT—AN ESSENTIAL ELEMENT IN ANIMAL NUTRITION—AUSTRALIAN INVESTIGATIONS. Science 85: 604–605.

(10) WHITE, HARVEY ELLIOTT.
1934. INTRODUCTION TO ATOMIC SPECTRA. 457 pp., illus. New York and London.

LABORATORY STUDIES ON TETRASTICHUS XANTHOMELAENAE ROND. AND TETRASTICHUS SP., TWO HYMENOPTEROUS EGG PARASITES OF THE ELM LEAF BEETLE [1] [2]

By P. A. BERRY

Assistant entomologist, Division of Forest Insect Investigations, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture

INTRODUCTION

Several attempts have been made to establish *Tetrastichus xanthomelaenae* Rond., an important egg parasite of the elm leaf beetle (*Galerucella xanthomelaena* (Schr.)), in the United States. The first shipments were made from France in 1908,[3] and up to 1932 occasional attempts were made to establish this species in the New England States. In 1932 importations on a larger scale were undertaken, and parasitized eggs were received from both France and Austria. These shipments were made early in the season in order to give the parasites access, upon their liberation in the United States, to the greatest possible number of elm leaf beetle eggs. Emergence of parasites began soon after the parasitized eggs arrived at the Melrose Highlands laboratory.

After many of the parasites had emerged, it was discovered that two species of *Tetrastichus* were present. One was *T. xanthomelaenae* and the other an unidentified species. Separation of the living specimens of these two species was difficult and was not considered to be practical in view of the large quantity of material received. It seemed advisable to hold all material in the laboratory until the status of the new species could be determined.

The percentage of *Tetrastichus* sp. occurring in the imported material has been low. A sample of 600 adults from Oberpullendorf, Austria, contained 29 *Tetrastichus* sp., or 4.8 percent. In a sample of 500 adults from Hyères, France, there were 17 *Tetrastichus* sp., or only 3.4 percent.

Apparently *Tetrastichus* sp. has always been present in much smaller numbers than *T. xanthomelaenae*. Marchal [4] made a number of observations on *T. xanthomelaenae* but did not encounter the new species in his work. In another paper,[5] in which he described *T. xanthomelaenae*, Marchal mentioned another parasite reported as attacking eggs of *Galerucella xanthomelaena*, but the record of the exact host is questionable and the brief notes on the parasite indicate that it was not *Tetrastichus* sp.

[1] Received for publication June 17, 1938; issued December, 1938.

[2] This study was conducted at the Melrose Highlands, Mass., laboratory of the Bureau of Entomology and Plant Quarantine during 1932 and 1935. The writer is indebted to C. W. Collins, in charge of the laboratory previous to 1935, and to R. C. Brown, in charge during 1935, for making these investigations possible, and to P. B. Dowden for helpful advice.

[3] HOWARD, L. O. THE IMPORTATION OF TETRASTICHUS XANTHOMELAENAE (ROND.). Jour. Econ. Ent. 1 281–289, illus. 1908.

[4] MARCHAL, P. OBSERVATIONS BIOLOGIQUES SUR UN PARASITE DE LA GALÉRUQUE DE L'ORME, LE TETRASTICHUS XANTHOMELAENAE (ROND.) (HYMÉN.). Bull. Soc. Ent. France 1905: 64–68. 1905.

[5] MARCHAL, P. IDENTIFICATION DU PARASITE DES OEUFS DE LA GALÉRUQUE DE L'ORME, TETRASTICHUS XANTHOMELAENAE (ROND.) (HYMÉN.). Bull. Soc. Ent. France 1905: 81–83, illus. 1905.

Parker [6] figured the egg, the first-instar larva, and the full-grown larva of *Tetrastichus* sp., but he reported it as *T. xanthomelaenae*. This is not surprising, for prior to 1932 only one species was known to attack the eggs of the elm leaf beetle.

EXPERIMENTS ON THE TWO PARASITES WHEN OCCURRING SEPARATELY AND WHEN IN COMPETITION

Experiments were undertaken to determine the status of each species of parasite when occurring separately, the effect that one would have upon the efficiency of the other when they were operating together, and whether either species acted as a secondary parasite upon the other species. For this purpose it was necessary to conduct several series simultaneously. In all the experiments the development was observed through daily dissections of a portion of the exposed material.

In the first series of experiments each species of parasite was reared separately from egg to adult on clusters of elm leaf beetle eggs collected from several localities in Massachusetts, and the life cycles and the principal habits of the two species were studied. *Tetrastichus xanthomelaenae* oviposited freely in the elm leaf beetle eggs, dissections showing that each egg contained from one to four eggs of the parasite. Only one individual, however, developed to maturity in a single host egg; the others succumbed in an early stage of development. The cause of the death of the excess young larvae where superparasitization occurred was not apparent. Adult males and females issued in about equal numbers from all the lots exposed. The average length of the developmental period from the time the eggs were laid until the adults issued was 16.9 days. The average life cycle of *Tetrastichus* sp. was found to be 17.5 days, or about 14 hours longer than that of *T. xanthomelaenae*. In other respects the development was the same in the two species.

In the second series of experiments several masses of elm leaf beetle eggs were exposed to a mixed lot of parasites containing equal numbers of mated females of each species. Dissections of a part of the material showed a high percentage of parasitization and considerable superparasitization. Frequently eggs and larvae of both species of parasites were found in the same host egg. Only one of the larvae developed to maturity, however. Sometimes it was one species and sometimes the other. Out of 200 dissected eggs that contained both species of larvae with only one larva living, there were 85 eggs in which the larvae of *Tetrastichus xanthomelaenae* remained alive and 115 in which the *Tetrastichus* sp. survived. The eggs that were allowed to develop to maturity produced 105 *T. xanthomelaenae* and 93 *Tetrastichus* sp. It is evident, therefore, that the chances of developing to maturity are about equal for the two species when they both attack at the same time.

In the third set of experiments egg masses were exposed first to one species of *Tetrastichus* and then to the other. Several egg masses were exposed to *T. xanthomelaenae* for 4 hours. This material was then divided into 10 lots, which were numbered from 1 to 10. Lot No. 1 was exposed to *Tetrastichus* sp. the day following exposure to

[6] PARKER, H. L. RECHERCHES SUR LES FORMES POST-EMBRYONNAIRES DES CHALCIDIENS. Ann. Soc Ent. France 93: [261]-379, illus. 1924.

T. xanthomelaenae, lot No. 2 the second day, and so on until the sixth day, when the parasites refused to oviposit in the parasitized eggs. Dissections of a number of samples showed that oviposition by *Tetrastichus* sp. in the parasitized eggs decreased after the second day and only a few of those offered on the fifth day were attacked. There were a few cases in which the *Tetrastichus* sp. survived where from 1 to 2 days intervened between the periods of attack. Where there was an interval of more than 2 days, *T. xanthomelaenae* survived in all cases observed.

In the experiments in which the order of attack by the parasites was reversed, the results were similar in that the species attacking first survived in much the greater numbers. Neither species, therefore, acted as a secondary parasite on the other.

CHARACTERS FOR SEPARATING THE EARLY STAGES AND ADULTS OF TETRASTICHUS XANTHOMELAENAE AND TETRASTICHUS SP.

Eggs and early-instar larvae of these two species of *Tetrastichus* may be readily separated, but no distinguishing characters have been found for separating the later instars or the pupae.

EGG

The egg of *Tetrastichus xanthomelaenae* (fig. 1, *D*) measures 0.23 mm in length and 0.075 mm at its greatest width. It is almost circular in cross section and slightly curved in shape. It is white and has a smooth surface. From the cephalic end, which is bluntly rounded, the egg thickens slightly for a short distance and then gradually tapers to a round caudal end.

The egg of *Tetrastichus* sp. (fig. 1, *B*) measures 0.22 mm in length and 0.06 mm at its greatest width. It is almost circular in cross section and slightly curved in shape. It is white and has a smooth, glistening surface. The cephalic end has a protuberance resembling a petiole. From this petiolar region the egg thickens abruptly to its greatest diameter, from which it tapers gradually to a blunt caudal end.

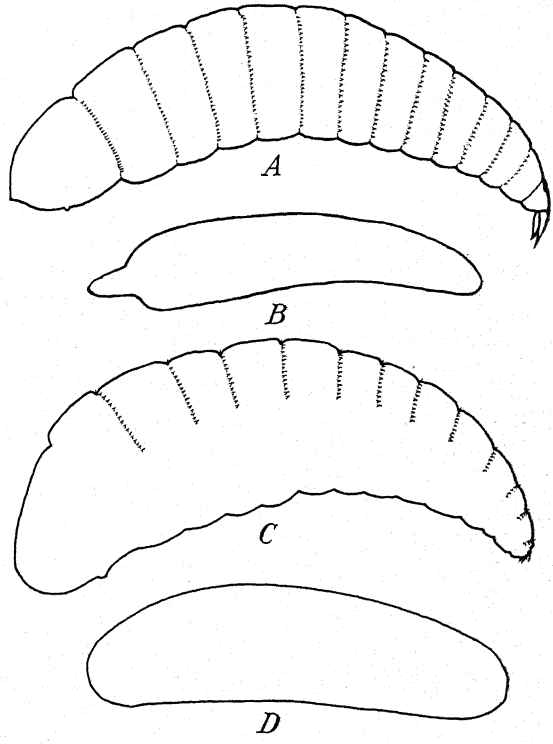


FIGURE 1.—*Tetrastichus* sp.: *A*, First-instar larva, and *B*, egg. *Tetrastichus xanthomelaenae: C*, First-instar larva, and *D*, egg.

LARVA

The first-instar larva of *Tetrastichus xanthomelaenae* (fig. 1, *C*), shortly after emerging from the egg, is 0.32 mm in length and 0.09 mm at its greatest width. The head is long and shaped like a thimble, but it is shorter and thicker than the head of *Tetrastichus* sp. There are 3 thoracic and 10 abdominal segments. The last segment is short and has a complete row of caudal hairs or spines, which are heavier than those on the other segments. On the posterior margins of each of the other segments there is a row of spines of about equal length extending across the dorsum and almost to the lateral margins. The young larva is hyaline, the midintestine being visible through the body walls.

The first-instar larva of *Tetrastichus* sp. (fig. 1, *A*), shortly after emerging from the egg, measures 0.37 mm in length and 0.09 mm at its greatest diameter. It is cylindrical in form, with diameter decreasing towards the posterior extremity. It has a long, thimble-shaped head and 3 thoracic and 10 abdominal segments. The last abdominal segment is terminated by two long, slightly curved hooks placed side by side. All segments except the last have rows of spines completely encircling the larva. The larva is hyaline.

ADULT

The characters for separating the adults of these two species of parasites, as described by B. D. Burks,[7] of the Illinois Natural History Survey, are as follows:

Tetrastichus xanthomelaenae (Rond.)—Thorax dark metallic blue-green, abdomen entirely black, with faint metallic luster; pedicel of antenna globose in both sexes; flagellum of the males bearing short setae; none longer than the third funicle segment; submarginal vein of forewing with one dorsal bristle.

Tetrastichus sp. "Y"—Thorax shining black, abdomen dark brown and yellow at base; pedicel of antenna slender, elongate, slightly enlarged at apex in both sexes; flagellum of male bearing long setae; those borne by the second funicle segment longer than the entire funicle; submarginal vein of forewing with two dorsal bristles.

LIBERATIONS AND RECOVERIES

Prior to 1932 from 2,000 to 2,500 adults of *Tetrastichus xanthomelaenae* had been liberated at various localities in New England and New Jersey. A large number of these were reared in the laboratory at Melrose Highlands, Mass.[8] From 1932 to 1935, inclusive, 35,234 adults were imported and liberated in the United States, as follows: Massachusetts, 8,445; Connecticut, 4,000; New Hampshire, 600; New York, 1,000; New Jersey, 2,484; District of Columbia, 3,950; Virginia, 2,750; Ohio, 5,000; and California, 7,005.

Several collections of elm leaf beetle eggs in Massachusetts and one in New Jersey have shown that the parasites reproduce in the field the same season in which they are liberated, but none have been recovered the following season. In 1933 collections of 449 egg masses from the sites of the 1932 liberations and in 1934 collections of 689 egg masses from the 1932–33 liberation points failed to produce a single parasite.

[7] Private communication.

[8] HOWARD, L. O., and FISKE, W. F. THE IMPORATION INTO THE UNITED STATES OF THE PARASITES OF THE GYPSY MOTH AND THE BROWN TAIL MOTH: A REPORT OF PROGRESS, WITH SOME CONSIDERATION OF PREVIOUS AND RECURRENT EFFORTS OF THIS KIND. U. S. Bur. Ent. Bull. 91, 344 pp., illus. 1911.

SUMMARY

In the course of attempts to establish the elm leaf beetle parasite *Tetrastichus xanthomelaenae* Rond. in the United States, another, unidentified, species of *Tetrastichus* was discovered in the imported material. A study of the two species was therefore undertaken in the laboratory to observe their development and parasitic habits, both when each species occurred separately and when the two were in competition. The characters distinguishing the two species were also determined.

The development was found to be similar in the two species, except that the life cycle of *Tetrastichus* sp. was slightly longer than that of *T. xanthomelaenae*. When superparasitism or multiple parasitism occurred, all but one larva in each host egg died in the early instars. Where the host was attacked by both species, the one attacking first had the better chance of surviving. Both species are therefore primary parasites.

The eggs and young larvae of the two parasites have definite characters by which they can be readily separated, but no distinguishing characters were found for the later instars or the pupae.

In all importations the percentages of adult *Tetrastichus* sp. emerging from the beetle eggs were low, never exceeding 5 percent.

EFFECT OF EXTERIOR TEMPERATURE UPON PRESS FLUID, SHEAR FORCE, AND COOKING LOSSES OF ROASTED BEEF AND PORK MUSCLES [1]

By ALICE M. CHILD, *associate professor of home economics*, and MARY J. SATORIUS, *instructor in home economics, Minnesota Agricultural Experiment Station* [2]

INTRODUCTION

In meat cookery, methods must be worked out by which palatability can best be retained and developed. Palatability is in large measure dependent upon the structure of the muscle tissue. The present work was undertaken to study the effect of exterior or oven temperature upon structure of meat when the two following mechanical devices were employed: The pressometer, used by Child and Fogarty (*4*),[3] which measures press fluid (defined as fluid consisting of moisture plus the soluble material plus the colloidal fraction pressed from muscle by the pressometer), and the Minnesota modification of the Warner-Bratzler shear-force apparatus which measures the force necessary to shear a sample of meat of given dimensions.

The study covered the effect of oven or exterior temperature upon press fluid, shear force, and cooking losses during the roasting of the following muscles: (1) Semitendinosus beef muscle heated to an internal temperature of 58° C. at oven temperatures of 125°, 150°, 175°, and 200°; (2) longissimus dorsi beef muscle heated to an internal temperature of 58° at constant oven temperatures of 150° and 200°, and at 150° after searing at 260°; and (3) longissimus dorsi pork muscle heated to an internal temperature of 84° at constant oven temperatures of 125°, 150°, and 175°, and at 150° after searing at 260°.

MATERIALS AND METHODS

Beef of high medium to good grade which had been ripened 12 days at 2° to 3° C. was obtained from a local packing plant. The semitendinosus or "eye" muscle from the bottom round had been found homogeneous by Child and Fogarty (*4*) so that two comparable roasts, weighing about 1.5 pounds each, could be obtained from each muscle, giving four comparable roasts from one animal. A series of 12 animals was studied, 48 roasts in all. The longissimus dorsi muscle had not yet been studied for homogeneity at the time of this experiment. Thus, from a series of 18 animals pairs of seventh-eighth standing ribs, weighing about 7 pounds each, were used for one comparison of external temperatures, and ninth-tenth ribs, weighing about 6 pounds each, were used for another, a total of 72 beef rib roasts.

Pork was obtained through the local packing plant and was ripened 4 days at 2° to 3° C. The longissimus dorsi muscle from center pork

[1] Received for publication February 1, 1938; issued December, 1938. Scientific Journal series, paper No. 580, Minnesota Agricultural Experiment Station.

[2] The authors gratefully acknowledge their indebtedness to George Steinacher for technical assistance in constructing the apparatus shown in figure 1, and to L. W. Neubauer, Agricultural Engineering Department, for the drawing.

[3] Italic numbers in parentheses refer to Literature Cited, p. 871.

loin containing four thoracic and four lumbar vertebrae had been found to be homogeneous,[4] so that the cut could be halved between the thoracic and lumbar vertebrae to give two comparable roasts weighing 1.8 pounds each. Thus, four comparable roasts from each of 12 animals or a total of 48 roasts were used.

ROASTING AND SAMPLING MEAT

All meat was cooked in electric laboratory ovens, controlled to 2° F. with regulators, by methods recommended by the cooking committee [5] of the cooperative meat investigation committee. Cooking data, i. e., losses, cooking time, and temperature rise after removal from the oven were recorded.

The adipose covering of the semitendinosus muscle was removed and the four comparable roasts were cooked to 58° C. at oven temperatures of 125°, 150°, 175°, and 200°. The meat was cooled to 40° for sampling. To better control biological variation the order of roasting was rotated so that one cut was not always cooked at the same temperature.

The seventh-eighth and ninth-tenth beef ribs were cooked to 58° C. One of each pair of seventh-eighth ribs was cooked at 150° constant temperature and compared to the other one which was cooked at 150° after searing for 20 minutes at 260°. One of each pair of ninth-tenth ribs was cooked at 150° and compared to the other one cooked at 200°. The meat was cooled to 40° for sampling.

The four comparable roasts from center pork loin were cooked to 84° C. at constant oven temperatures of 125°, 150°, and 175°, and at 150° after searing for 20 minutes at 260°. The roasts were rotated as for the semitendinosus muscle. The meat was cooled to 80° for sampling.

After cooling, the fat and bone on the roast were removed and the muscle was halved through the thermometer hole perpendicular to the muscle fibers. A slice 1.25 cm thick was cut for press-fluid determinations, and from this two samples, on either side of the thermometer hole, were taken parallel to the muscle fibers with a borer 1.25 cm in diameter. The other half of the roast was used for tenderness determinations and from it two samples, about 3.8 cm long, were taken parallel to the muscle fibers with a borer 2.54 cm in diameter.

PRESS-FLUID DETERMINATION

The two samples of meat cut for press-fluid determinations were weighed to the nearest tenth of a milligram. Each sample, weighing about 1.5 g, was wrapped in filter cloth and pressed 10 minutes under a pressure of 250 pounds by the pressometer, which was standardized by Child and Baldelli (*3*). The difference in weight of the meat before and after pressing was referred to as the weight of the press fluid.

SHEAR-FORCE DETERMINATION

A modification of the Warner-Bratzler [6] shear-force apparatus was used to measure shear force, and figure 1 shows its mechanism. Each

[4] CHILD, ALICE M., and SATORIUS, MARY J. A STUDY OF SAMPLING FOR MEAT RESEARCH. Unpublished data, Minnesota Experiment Station. 1937.

[5] ALEXANDER, LUCY M., CLARK, N. G., and HOWE, P. E. METHODS OF COOKING AND TESTING MEAT FOR PALATABILITY. Supplement to National Project Cooperative Meat Investigations. U. S. Dept. Agr., Bur. Home Econ. and Bur. Anim. Indus. 36 pp., illus. Revised February 1933. Mimeographed.

[6] BRATZLER, T. J. MEASURING THE TENDERNESS OF MEAT BY MEANS OF A MECHANICAL SHEAR. Unpublished data, Kansas State College. 1932.

sample cut for shear-force determination was inserted in a triangular opening in a dull blade 1 mm in thickness. Shearing bars were started downward at the rate of 23.5 cm per minute by an automatic switch and electric motor and the pounds of force necessary to shear the

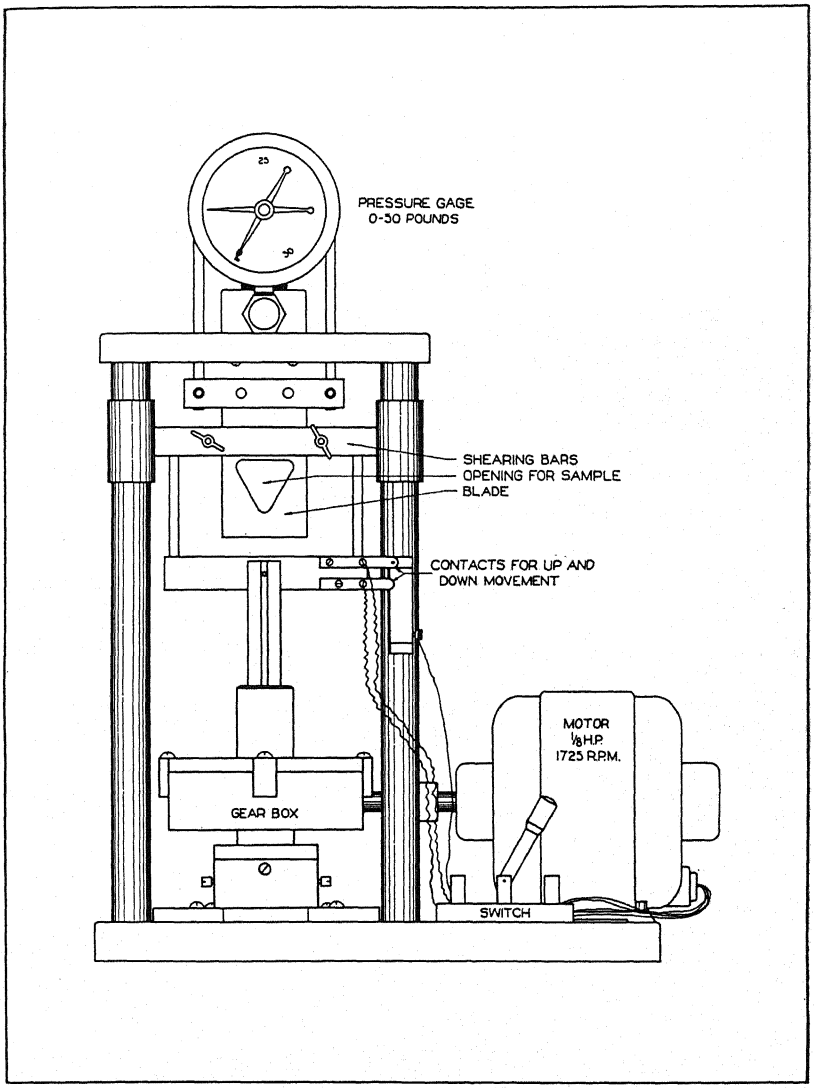


FIGURE 1.—Diagram of shear-force apparatus, showing detail of working mechanism.

meat were recorded to the nearest one-fourth pound on the gauge. Three determinations, one at the center and one at the center of each half, were made on each of the two samples; the six readings were averaged for each roast.

EXPERIMENTAL DATA

SEMITENDINOSUS MUSCLE

The roasts cooked to 58° C. at 125°, 175°, and 200° were compared to those cooked at 150°, since this oven temperature has been generally accepted. Press fluid and shear force were not significantly affected by exterior temperature, as shown by the Fisher (7) *t* test (table 1). The values for *t* did not exceed 1.3, which has a corres-

TABLE 1.—*Percentage press fluid, total losses, and pounds of shear force for semitendinosus beef muscle heated to an internal temperature of 58° C. at oven temperatures of 125°, 150°, 175°, and 200°*

Series No.	Press fluid				Total losses				Shear force			
	125° C.	150° C.	175° C.	200° C.	125° C.	150° C.	175° C.	200° C.	125° C.	150° C.	175° C.	200° C.
	Per-cent (1)	*Per-cent* (2)	*Per-cent* (3)	*Per-cent* (4)	*Per-cent* (1)	*Per-cent* (2)	*Per-cent* (3)	*Per-cent* (4)	*Pounds* (1)	*Pounds* (2)	*Pounds* (3)	*Pounds* (4)
1	55.63	53.29	56.05	59.27	14.84	14.51	16.78	19.49	11.6	12.1	14.0	17.7
2	54.33	52.93	55.15	54.13	14.93	14.19	13.62	16.16	15.9	13.1	11.1	13.5
3	57.01	55.89	55.43	54.93	12.47	14.78	16.32	18.61	17.5	14.5	15.9	14.5
4	52.91	54.61	53.45	55.64	14.54	15.83	18.42	17.01	12.2	12.5	11.7	10.9
5	56.92	57.53	55.25	54.57	14.53	14.85	16.41	18.12	10.9	12.3	15.3	15.1
6	56.11	54.18	53.72	49.68	14.02	14.66	16.84	20.57	11.6	9.1	13.7	16.7
7	57.12	58.08	55.96	57.97	13.14	14.81	20.80	19.22	14.9	12.9	12.9	11.5
8	56.83	56.51	54.35	52.31	15.37	16.25	20.26	19.65	7.5	10.5	11.9	12.3
9	54.43	54.89	54.73	57.91	11.06	12.01	18.95	16.97	10.2	10.6	11.3	8.3
10	52.89	54.23	55.14	53.19	13.68	13.94	12.92	17.09	16.3	12.3	10.5	17.1
11	51.29	53.91	56.36	55.91	14.04	14.04	14.39	16.46	15.1	15.9	11.3	10.9
12	54.64	55.18	56.27	55.73	14.55	14.75	14.57	17.66	11.7	10.7	10.7	14.7
Mean	55.01	55.10	55.15	55.10	13.93	14.55	16.69	18.08	12.9	12.2	12.5	13.6
	(1) and (2)		(3) and (2)	(4) and (2)	(1) and (2)		(3) and (2)	(4) and (2)	(1) and (2)		(3) and (2)	(4) and (2)
	0.21		0.10	0.00	2.50		2.97	8.97	1.21		0.45	1.30
P	>.56		>.56	>.56	.03		.01	<.01	.25		>.56	.22

ponding value of $P=0.22$. This value is above the 5-percent level for significance or the 1-percent level for high significance. Total losses, however, were found to increase with each increment in exterior temperature; the increase was due to evaporation losses only. The values for *t* ranged from 2.50 to 8.97.

It has furthermore been shown [7] that when semitendinosus muscle is heated to 58° C. by dry heat, by cooking in a covered pan and by adding water in a covered pan, press fluid and shear force are not affected but cooking losses are increased by the moist heat.

A correlation of −0.05 was found between press fluid and total cooking losses for semitendinosus muscle heated to 58° C., a value which is not significant. Child and Fogarty (4) also found an insignificant correlation of −0.18 between press fluid and total losses for semitendinosus muscle heated to 58°.

STANDING BEEF RIBS

Press fluid did not vary significantly among beef ribs heated to 58°C. at constant temperatures of 150° and 200° and at 150° after searing at 260° (table 2). Values for *t* were not higher than 0.93, which give values for *P* far above the 5-percent level of significance. Searing did not affect shear force, but a significantly greater number of pounds

[7] NIENOW, I. EFFECT OF MOIST HEAT ON SEMITENDINOSUS MUSCLE. Unpublished data, Minnesota Agricultural Experiment Station. 1937.

of force were required to shear the ribs heated at 200° than those heated at 150°, a value for t of 2.99 being obtained. Cover (*6*) found beef ribs cooked well-done at 225° less tender when subjectively judged than those cooked at 125°, a finding which she thought due to the short cooking period at 225°.

TABLE 2.—*Percentage press fluid, total losses, and pounds of shear force for standing beef ribs heated to an internal temperature of 58° C. at constant oven temperatures of 150° and 200°, and at 150° following searing at 260°*

Series No.	Press fluid				Total losses				Shear force			
	Seared,[1] 150° C.	Constant, 150° C.	200° C.	150° C.	Seared,[1] 150° C.	Constant, 150° C.	200° C.	150° C.	Seared,[1] 150° C.	Constant, 150° C.	200 °C.	150° C.
	Percent (1)	*Percent* (2)	*Percent* (1)	*Percent* (2)	*Percent* (1)	*Percent* (2)	*Percent* (1)	*Percent* (2)	*Pounds* (1)	*Pounds* (2)	*Pounds* (1)	*Pounds* (2)
1	49.59	49.89	50.93	48.22	14.83	12.42	15.32	11.91	19.0	16.0	19.7	22.6
2	47.26	46.89	48.90	41.76	-------	-------	22.52	15.41	21.8	22.4	20.4	18.1
3	46.95	50.95	47.88	46.67	16.43	14.65	25.88	15.48	21.1	24.5	22.7	26.0
4	47.20	49.93	50.36	50.60	19.22	15.17	25.60	17.40	18.2	19.4	22.0	16.4
5	50.17	54.80	48.72	56.51	15.93	11.31	24.31	11.55	22.3	20.1	20.0	18.5
6	48.77	50.15	46.83	49.08	19.16	15.84	32.87	17.65	27.3	21.6	27.4	22.6
7	57.61	49.00	45.91	53.41	16.01	17.26	29.60	18.50	24.0	19.3	20.2	19.3
8	50.73	51.31	47.83	47.73	13.39	14.65	18.55	12.24	18.9	20.2	25.7	17.6
9	44.95	46.59	48.42	48.97	16.84	12.70	21.11	13.85	30.0	31.3	17.3	11.7
10	48.85	44.95	45.48	46.92	15.76	15.74	18.45	11.36	21.5	25.7	29.1	21.2
11	54.49	54.67	52.35	49.52	14.37	12.57	16.75	10.34	22.2	20.5	16.8	14.8
12	47.95	51.49	50.37	50.07	15.23	13.53	19.10	12.63	21.5	25.5	16.9	21.7
13	50.24	49.29	46.22	49.26	16.81	15.35	22.93	14.14	30.5	31.4	31.4	17.0
14	43.81	47.76	48.52	51.41	19.28	16.22	19.71	13.60	25.1	28.0	24.0	17.7
15	43.63	46.71	51.79	49.41	16.96	15.63	17.21	12.62	20.9	22.3	34.1	21.3
16	42.24	48.08	48.70	50.33	16.49	16.06	21.99	11.10	22.6	21.2	23.3	26.1
17	45.79	41.37	49.97	53.73	17.19	15.67	-------	-------	20.8	21.4	22.7	16.7
18	49.65	47.31	51.15	51.03	16.69	14.02	19.44	13.49	38.8	32.1	27.8	24.8
Mean	48.33	48.95	48.91	49.70	16.50	14.64	21.84	13.72	23.7	23.5	23.4	19.7
	(1) and (2)		(1) and (2)		(1) and (2)		(1) and (2)		(1) and (2)		(1) and (2)	
t	0.72		0.93		4.47		10.96		0.24		2.99	
P	.48		.37		<.01		<.01		>.56		<.01	

[1] 20 minutes at 260° C.

Cooking losses were increased both by a temperature of 200° C. and by initial searing (table 2), values for t of 10.96 and 4.47, respectively, being found. It has also been found by Cline (*5*) that beef cooked to 58° C. suffers more losses when cooked at high oven temperatures and when seared than at low constant temperatures and by Alexander (*1*) that high oven temperatures increase losses.

Furthermore, it has been shown [8] that beef ribs cooked to 58° C. in covered and uncovered pans do not differ in press fluid or shear force but cooking losses are increased by covering.

PORK LOINS

The pork loins cooked to 84° C. at constant temperatures of 125° and 175°, and at 150° following searing at 260°, were compared to those cooked at 150°. Press fluid of pork loin was not affected by different oven temperatures (table 3), for values of t did not exceed 1.67, which corresponds to $P=0.12$, an insignificant value. Shear force was not affected by oven temperature except that less force was required to shear the loins cooked at 125° than those cooked at 150°, a value for t

[8] NIENOW, I. See footnote 7, preceding page.

of 3.50 being obtained. The long cooking period of 138 minutes per pound at 125° as compared to 83 minutes at 150° may explain this difference. Only 57 minutes per pound were required at 175° and 62 at 150° following searing at 260°.

Total cooking losses of pork loin heated to 84° C. were not affected by constant exterior temperatures of 125°, 150°, and 175°, or by a temperature of 150° following searing, for all values of t were 0.76 or below. Alexander (*2*) found that cooking losses were not affected by oven temperatures when leg of lamb was cooked to 84°, but when cooked to 76° losses were increased by higher oven temperatures.

A significant correlation, −0.45, was found between press fluid and cooking losses of pork loin cooked to 84° C.

TABLE 3.—*Percentage press fluid, total losses, and pounds of shear force for pork loins heated to an internal temperature of 84° C. at constant oven temperatures of 125°, 150°, 175°, and at 150° following searing at 260°*

Series No.	Press fluid				Total losses				Shear force			
	125° C.	150° C.	175° C.	Seared [1] 150° C.	125° C.	150° C.	175° C.	Seared [1] 150° C.	125° C.	150° C.	175°C.	Seared [1] 150° C.
	Pct. (1)	*Pct.* (2)	*Pct.* (3)	*Pct.* (4)	*Pct.* (1)	*Pct.* (2)	*Pct.* (3)	*Pct.* (4)	*Lbs.* (1)	*Lbs.* (2)	*Lbs.* (3)	*Lbs.* (4)
1	38.11	42.44	45.45	51.44	20.42	22.35	23.60	17.53	6.1	9.6	6.5	9.3
2	40.59	40.67	49.23	43.85	21.24	25.56	22.26	25.44	6.5	7.6	7.6	9.5
3	45.39	44.85	47.43	50.21	20.66	26.53	20.70	22.25	8.1	8.3	8.5	8.5
4	35.96	41.93	42.22	38.78	21.57	20.41	22.17	26.47	9.3	10.3	10.6	12.0
5	42.33	43.12	42.14	49.21	17.93	20.15	22.50	18.81	5.5	10.1	8.6	8.1
6	29.58	48.48	38.03	42.92	26.13	23.51	25.64	22.50	5.6	8.0	8.5	8.5
7	37.59	34.60	41.18	41.44	26.50	25.53	24.76	24.02	6.5	8.5	6.3	7.6
8	39.85	37.54	39.93	37.05	18.66	27.06	27.91	25.85	6.5	9.8	9.0	13.0
9	42.98	44.75	47.55	40.89	29.22	24.32	23.35	26.81	4.3	4.3	7.8	6.8
10	33.48	38.18	34.97	39.46	31.13	20.93	22.61	20.45	6.1	7.5	7.1	6.6
11	44.06	47.72	37.67	44.06	24.52	18.36	22.39	23.71	7.8	7.5	6.6	7.6
12	45.51	44.78	44.06	38.22	21.56	17.12	21.78	23.83	6.3	6.3	7.0	8.8
Mean	39.62	42.42	42.49	43.13	23.29	22.65	23.31	23.14	6.5	8.1	7.8	8.9
	(1) and (2)		(3) and (2)	(4) and (2)	(1) and (2)		(3) and (2)	(4) and (2)	(1) and (2)		(3) and (2)	(4) and (2)
t	1.67	-------	0.04	0.46	0.41	-------	0.76	0.44	3.50	-------	0.64	1.50
P	.12	-------	>.56	>.56	>.56	-------	.46	>.56	<.01	-------	.53	.16

[1] 20 minutes at 260° C.

SUMMARY AND CONCLUSIONS

Semitendinosus beef muscle heated to an internal temperature of 58° C. at oven temperatures of 125°, 150°, 175°, and 200° did not differ in press fluid or shear force. Cooking losses were increased with increased exterior temperature because of losses from evaporation.

Standing beef ribs heated to an internal temperature of 58° C. at constant oven temperatures of 150° and 200°, and at 150° following searing at 260°, showed no differences in press fluid or shear force except that more force was required to shear those cooked at 200° than those cooked at 150°. Cooking losses were greater at 200° and at 150° following searing than at 150° constant temperature.

Pork loin cooked to an internal temperature of 84° C. at constant oven temperatures of 125°, 150°, and 175°, and at 150° following searing at 260° did not differ in press fluid or shear force except that the loin cooked at 125° required less force to shear than that cooked at 150°. Cooking losses were not affected by exterior temperature.

From the data obtained the following conclusions may be drawn:

Exterior or oven temperatures of 125° to 200° C. do not affect press fluid of roasted meat, and only at the extreme ends of the temperature range is shear force affected. More pounds of force may be required to shear roasts cooked at temperatures of 200° or higher, and fewer pounds of force to shear those cooked at temperatures of 125° or lower. However, at the low temperatures, the cooking time is very long, especially for well-done meat.

High exterior temperatures and searing increase cooking losses in rare meat but not in well-done meat.

LITERATURE CITED

(1) ALEXANDER, LUCY M.
1930. SHRINKAGE OF ROAST BEEF IN RELATION TO FAT CONTENT AND COOKING TEMPERATURE. Jour. Home Econ. 22: 915–922, illus.

(2) ——— and CLARK, NANCY GRISWOLD.
1934. SHRINKAGE AND HEAT PENETRATION DURING THE ROASTING OF LAMB AND MUTTON AS INFLUENCED BY CARCASS, GRADE, RIPENING PERIOD AND COOKING METHOD. U. S. Dept. Agr. Tech. Bull. 440, 26 pp., illus.

(3) CHILD, ALICE M., and BALDELLI, MARY.
1934. PRESS FLUID FROM HEATED BEEF MUSCLE. Jour. Agr. Research 48: 1127–1134, illus.

(4) ——— and FOGARTY, J. AGNES.
1936. EFFECT OF INTERIOR TEMPERATURES OF BEEF MUSCLE UPON THE PRESS FLUID AND COOKING LOSSES. Jour. Agr. Research 51: 655–662.

(5) CLINE, JESSIE ALICE, TROWBRIDGE, E. A., FOSTER, M. T., and FRY, HAZEL ELINOR.
1930. HOW CERTAIN METHODS OF COOKING AFFECT THE QUALITY AND PALATABILITY OF BEEF. Mo. Agr. Expt. Sta. Bull. 293, 40 pp., illus.

(6) COVER, SYLVIA.
1937. THE EFFECT OF TEMPERATURE AND TIME OF COOKING ON THE TENDERNESS OF ROASTS. Tex. Agr. Expt. Sta. Bull. 542, 61 pp., illus.

(7) FISHER, R. A.
1932. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 4, rev. and enl., 307 pp., illus. Edinburgh and London.

111469—38——6

U. S. GOVERNMENT PRINTING OFFICE: 1938

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 57 WASHINGTON, D. C., DECEMBER 15, 1938 No. 12

RESPIRATION AND LACTIC ACID PRODUCTION BY A FUNGUS OF THE GENUS RHIZOPUS[1]

By SELMAN A. WAKSMAN, *microbiologist*, and J. W. FOSTER, *research assistant*, *Department of Soil Chemistry and Microbiology, New Jersey Agricultural Experiment Station*

INTRODUCTION

Lactic acid formed in living systems is usually considered the characteristic product of the anaerobic or fermentative phase of metabolism. Among the micro-organisms, this acid was formerly believed to be associated with the activities of certain specific bacteria, found among several groups of anaerobic organisms, comprising both rod-shaped and spherical forms. Recently, however, it has been definitely established that this acid is also produced during the growth of certain fungi belonging largely to the Mucorales. Although these fungi have long been known as capable of growing both under aerobic and anaerobic conditions, the final product of their anaerobic metabolism has usually been found to be an alcohol. On the other hand, lactic acid is produced by some of these fungi in an aerobic environment, with the organisms forming a pellicle on the surface of the medium; even when the cultures are thoroughly aerated, the acid still is formed. One may, therefore, be justified in asking whether the lactic acid thus produced by the fungi is a result of the aerobic oxidation of the carbohydrate or whether it is due to an anaerobic phase of their metabolism. In other words, can lactic acid be a product of normal respiration, that is, of the oxidative activities of the fungi, or do the fungi possess, like animals and higher plants, a fermentative or glycolytic stage in their metabolism.

The following experiments deal with the nutrition of a fungus belonging to the genus *Rhizopus*,[2] its ability to produce lactic acid from different carbohydrates, the mechanism of production of this acid, and the role of this process in the metabolism of the fungus.

METHODS

It has previously been shown (*30*)[3] that the species of *Rhizopus* used in the work reported in this paper grows readily on simple inorganic media containing glucose or starch as sources of energy and carbon for cell synthesis. When $CaCO_3$ was present in the medium, abundant lactic acid was produced and accumulated in the medium. In the absence of a neutralizing agent, lactic acid was formed but it accumulated only until the limiting reaction was reached, namely, about pH 4.0; however, growth of the organism continued even after this reaction had been attained.

In order to measure the evolution of CO_2 during the growth of the organism, KOH or NaOH solutions were used in some experiments

[1] Received for publication Mar. 21, 1938. Issued February 1939. Journal series paper of the New Jersey Agricultural Experiment Station, Department of Soil Chemistry and Microbiology.

[2] Of the group *R. arrhizus* or *R. nodosus*.

[3] Italic numbers in parentheses refer to Literature Cited, p. 898.

to neutralize the acid formed. The addition of the alkali was begun after growth of the fungus was well established. Because of the detrimental effect of even a slight excess of the alkali on germination of the fungus spores, care was exercised not to render the medium too alkaline. An excess of alkali can be avoided by the addition of an indicator to the medium; bromcresol purple, at the rate of 1 cc of a 0.5-percent solution per 100 or 200 cc of medium, was found to be suitable for this purpose. The sterile alkali solution, 0.25 to 1.0 N, was added directly to the culture until a change in color at about pH 6.0 had been attained. An arrangement for the growth of the organism, for the neutralization of the acid produced, and for measurement of the CO_2 liberated in the process of growth is shown in figure 1. The CO_2 was absorbed in a standard $Ba(OH)_2$ solution and the excess barium titrated with standard oxalic acid solution.

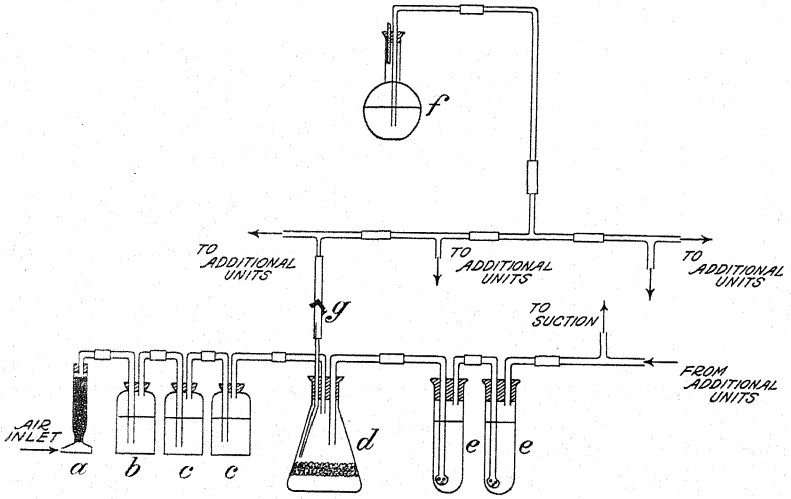


FIGURE 1.—Apparatus for neutralization of acid formed and for measurement of CO_2 evolved in the growth of fungi: Sterile 0.25 N KOH is introduced from reservoir (*f*) into culture flask (*d*) by means of stopcock (*g*). The flask is shaken gently in rotary fashion during the addition of alkali until the desired pH value is obtained as indicated by color change of indicator in culture solution. The air is freed of CO_2 and washed by passage through a soda lime column (*a*), 10-percent H_2SO_4 (*b*), and water (*c*). The CO_2 from culture flask (*d*) is aspirated into a series of tubes (*e*) containing standard $Ba(OH)_2$ solution.

The medium most commonly used in these investigations consisted of 2 g of $(NH_4)_2SO_4$, 0.5 g of K_2HPO_4, 0.5 g of $MgSO_4.7H_2O$, and 0.1 g of $Fe_2(SO_4)_3$ per liter of distilled water. Potato starch or glucose was usually used in concentrations of 2.5 percent. Ordinarily, the medium was distributed in Erlenmeyer flasks, but for respiration studies, round-bottom long-necked flasks were used. Incubation took place at 28° C. The cultures were allowed to remain undisturbed for 2 days; sterile $CaCO_3$ was then added, equal to the amount of carbohydrate added to the culture, and the flasks were shaken by hand, once or twice daily, for a minute or two. In the aeration studies, the alkali was added once every day and the culture well shaken during the addition of the alkali solution.

At the end of different periods of incubation, the cultures were removed and filtered through paper. The pellicles from the $CaCO_3$-free cultures were collected, dried at 100° C., weighed, and analyzed for total nitrogen and ash. When $CaCO_3$ was present in the medium, the pellicles were first washed with a dilute HCl solution and water. It was later found that an approximate determination of the growth of the organism could be obtained by washing the pellicle with hot water, analyzing for total nitrogen, and calculating the growth on the basis of the nitrogen in the pellicle. This calculation was especially useful for cultures containing calcium carbonate, which tended to give erroneously high weights. The filtrate and washings were made up to volume and analyzed for sugar by the Bertrand method, for ammonia by distillation, and for lactic acid by evaporation of an aliquot portion, drying, weighing, igniting, and reweighing. The lactic acid content was calculated from the amount of CaO or K_2O in the ash. This method of determining lactic acid is not strictly accurate, but it was regarded as being suitable for the experiments reported here, where, for the most part, marked changes in acid production were obtained as a result of the different treatments. Lactic acid is by far the chief acid produced by this organism; fumaric acid was found to be present in most cultures amounting up to 3 percent of the sugar consumed; small amounts of other unidentified acidic constituents were also found. Numerous analyses of the alcohol-precipitated salt from the cultures always yielded values for calcium content which checked closely with that for lactic acid.

The amounts of the other minerals left in the culture were negligible, having been largely assimilated by the pellicle. When ammonium sulphate was used as a source of nitrogen, allowance was made for the calcium sulphate produced as a result of the assimilation of the ammonia by the organism. The calcium sulphate production was found to be roughly parallel to the ammonia consumption. The latter was either calculated from the residual ammonia or from the total nitrogen in the pellicle.

In order to establish whether the acid thus produced by the fungus is primarily lactic, a quantity of the filtrate was evaporated to a small volume and the calcium lactate precipitated with alcohol. The precipitate was washed with alcohol, dried, weighed, and ashed. The CaO obtained corresponded closely with that commonly obtained from the calcium salt of lactic acid. The zinc salt was also prepared and analyzed for ZnO and for rotation. Here again the values checked closely with those for *d*-lactic acid. This procedure was repeated frequently during these experiments and always yielded similar results. When cultures were to be neutralized with KOH, a measured quantity of $CaCl_2$ was added before concentration in order to obtain the calcium salt. It was later found that upon the addition of alcohol, a small amount of a precipitate, consisting largely of phosphates and sulphates of calcium and magnesium, formed immediately. When this was rapidly removed by filtration and the liquid allowed to remain for 24 to 48 hours, the final precipitate was practically pure calcium lactate.

When starch was used as a source of carbohydrate, the residual starch of dextrin was determined by diluting an aliquot portion of the filtrate with water, making 2 percent acid by the addition of HCl, heating for 15 minutes at 15 pounds pressure, neutralizing, and deter-

mining the reducing sugar. Allowance was made for the sugar found directly in the culture.

GROWTH OF RHIZOPUS AND PRODUCTION OF LACTIC ACID

In the first experiment, 2 percent of raw potato starch was used as a source of carbohydrate. A large number of flasks were prepared so that several could be removed at different intervals. The total incuba-

TABLE 1.—*Carbohydrate decomposition and lactic acid production by Rhizopus and growth and nitrogen content of pellicle during incubation for various periods*

[Results on the basis of 1 liter of medium]

Period of incubation (days)	Starch [1] left, as sugar	Sugar present	Total carbohydrate consumed, as sugar	Lactic acid produced [2]	Total CO_2 liberated as C	NH_4 N consumed	Pellicle	
							Weight	Nitrogen content
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
0	18,920	0	0	0	----------	0	----------	----------
1	18,580	0	340	0	----------	----------	----------	----------
2	17,000	540	1,380	0	100	17	----------	----------
3	7,490	6,340	5,090	3,170	293	39	420	21
4	[3] 3,930	4,720	10,270	8,350	490	47	590	30
5	[3] 1,100	3,740	14,080	11,710	670	59	----------	----------
7	[3] 400	1,280	17,240	14,720	941	73	[4] 740	37
8	----------	300	18,620	14,820	1,163	82	[4] 920	46
10	----------	60	18,860	13,340	1,522	78	1,090	56
12	0	0	18,920	12,770	1,710	94	----------	----------
15	0	0	18,920	----------	1,810	98	[4] 1,480	74
17	0	0	18,920	13,310	1,881	97	----------	----------
24	0	0	18,920	13,220	1,946	95	[4] 1,780	89

[1] Including dextrin.
[2] Calculated from CaO in ash.
[3] No starch was left at 4 days; excess carbohydrate above sugar is due to dextrin.
[4] Calculated from nitrogen content.

tion period was 29 days. The results, presented in table 1, show that the starch was at first rapidly hydrolyzed by the organism to sugar, and only the sugar was utilized for lactic acid production. Within 4 days all the starch had disappeared, although it had not all been converted to sugar. A part of it was left in the medium in the form of dextrin and could be converted to sugar by acid treatment. The pellicle was found to contain approximately 5 percent of nitrogen.

During the early stages of growth of the organism, the sugar consumed, calculated as carbon, could not be fully accounted for by the sum of carbon in the pellicle, in the lactic acid, and in the carbon dioxide. After 3 days, for example, 5,090 mg of carbohydrate had disappeared, the equivalent of 2,036 mg of carbon. The three products accounted for only 1,719 mg of carbon, the carbon content of the pellicle being taken as 50 percent. Table 2 shows the carbon relationships at the different stages of development of the fungus.

TABLE 2.—*Carbon relationships at different stages of development of a fungus of the genus Rhizopus*

Period of incubation (days)	Carbohydrate consumed per liter of medium, as carbon	Carbon accounted for in lactic acid, pellicle, and CO_2	Period of incubation (days)	Carbohydrate consumed per liter of medium, as carbon	Carbon accounted for in lactic acid, pellicle, and CO_2	Period of incubation (days)	Carbohydrate consumed per liter of medium, as carbon	Carbon accounted for in lactic acid, pellicle, and CO_2
	Milligrams	*Milligrams*		*Milligrams*	*Milligrams*		*Milligrams*	*Milligrams*
----------	2,036	1,719	7----------	6,896	7,043	10----------	7,544	7,263
----------	4,108	3,973	8----------	7,448	7,383	24----------	7,568	7,984

The difference in the carbon balance during the early stages of growth is believed to have been due to the formation of an intermediary compound which was later converted to lactic acid, cell substance, CO_2, or to all of these. Subsequent experiments demonstrated that this was actually the case; volatile intermediary products were demonstrated.

The results of this experiment, and of other experiments reported previously (*29*), show that lactic acid production attains a maximum in 7 to 8 days, at 28° C.; a period which corresponds with that required for the complete disappearance of the carbohydrate. A detailed analysis of the course of growth of the fungus, consumption of glucose, production of lactic acid, and evolution of CO_2 is presented graphically in figure 2. The results of the previous experiment with starch as the substrate were fully confirmed.

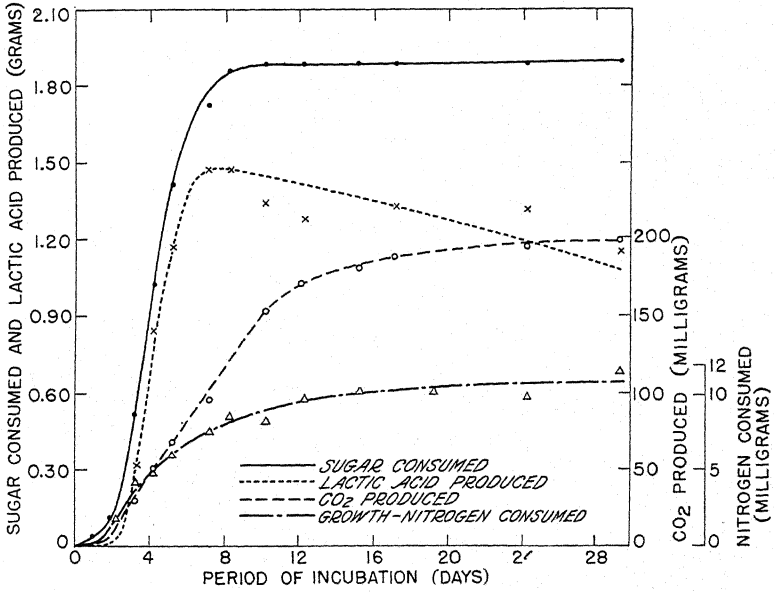


FIGURE 2.—Course of carbohydrate consumption, lactic acid production, CO_2 evolution, and growth of *Rhizopus*.

Assuming that lactic acid and CO_2 are the only final products of the reaction and leaving out of consideration the various intermediary products that are known to be formed, two possible explanations of the mechanism of lactic acid production by this fungus remain:

Skeleton reactions

(1) $$C_6H_{12}O_6 \rightarrow \underset{\text{(lactic acid)}}{C_3H_6O_3} + \underset{\text{(Unknown compound)}}{C_3H_6O_3};\ C_3H_6O_3 \xrightarrow{+3O_2} 3CO_2 + 3H_2O$$

(2) $$C_6H_{12}O_6 \xrightarrow{+3O_2} \underset{\text{(lactic acid)}}{C_3H_6O_3} + 3CO_2 + 3H_2O$$

In reaction (1), the ratio of $\frac{\text{lactic acid carbon}}{CO_2 \text{ carbon}}$ may be 1:1 or >1:1; the latter is possible when less than half of the lactic acid homologue is further oxidized. In reaction (2), the proportion of lactic acid carbon to CO_2 carbon cannot be greater than 1:1. By analyzing the results reported in table 1 for the 3-, 4-, 5-, 7-, 8-, and 24-day periods, the relations shown in table 3 are obtained.

TABLE 3.—*Analysis of the results presented in table 1 for the 3-, 4-, 5-, 7-, 8- and 24-day periods to present lactic acid-carbon dioxide ratios*

[Results on the basis of 1 liter of medium]

Period of incubation (days)	Carbohydrate C	Lactic acid C	CO_2C	Carbohydrate C / (lactic acid C)	Lactic acid C / (CO_2C)
	Milligrams	*Milligrams*	*Milligrams*		
3	2,040	1,220	290	1.7:1	4.2:1
4	4,110	3,190	490	1.3:1	6.5:1
5	5,630	4,580	670	1.2:1	6.8:1
7	6,900	5,730	940	1.2:1	6.1:1
8	7,450	5,760	1,160	1.3:1	5.0:1
24	7,570	5,290	1,950	1.4:1	2.7:1

The ratio of lactic acid C to CO_2C is always greater than 1:1. It ranges from 4.2:1 to 6.8:1, with an average for the five determinations during the early active stages of growth of 5.7:1. An analysis of the relation between carbohydrate consumed and lactic acid produced shows also a ratio greater than 1:1. These results point definitely to reaction (1) as the reaction concerned in the process of lactic acid production by *Rhizopus*. This reaction can be developed further to show that in addition to the lactic acid, an intermediary product is formed, which can be partly oxidized to CO_2 and partly transformed to lactic acid:

$$nC_6H_{12}O_6 = \underset{\text{Lactic acid}}{nC_3H_6O_3} + \underset{\text{Lactic acid}}{nC_3H_6O_3} + O_2 + 3CO_2 + 3H_2O$$

In the foregoing experiment, there was, after 24 days' growth, 5,290 mg of lactic acid carbon as compared with 1,950 mg of CO_2 carbon. At that time the ratio of carbohydrate carbon to lactic acid carbon was $\frac{7,570}{5,290}$, or 1.4:1, and the ratio of lactic acid carbon to CO_2 carbon was $\frac{5,290}{1,950}$, or 2.71:1. These ratios tend to approach the requirements for reaction (1). On the basis of the results of this experiment it may be concluded that *Rhizopus* breaks down the sugar first to some intermediate product, that part of this product is oxidized to CO_2, supplying the necessary energy for the growth of the organism, part is utilized for cell synthesis, and part is transformed to lactic acid. The balance between these three transformation products can be readily influenced by the conditions of growth of the organism. It will later be shown that the presence of different elements, notably salts of heavy metals, influence growth and lactic acid production differently.

In order to demonstrate quantitatively that the acid produced in the different stages of growth of the organism is lactic acid or predominantly so, the filtrates from several flasks of the organism grown on the above medium were collected after 7, 17, and 28 days' growth, concentrated on a water bath, and the calcium lactate precipitated with alcohol, washed with alcohol, and dried. The filtrate was further concentrated to a small volume and again precipitated with alcohol. The two precipitates were then combined and analyzed. The actual yields of calcium lactate obtained by precipitation were compared (table 4) with the amounts of lactic acid as calculated from the CaO content

TABLE 4.—*Purity and yield of acid produced by Rhizopus as compared with calculated yields after 7, 17, and 28 days of growth*

[Results on the basis of 1 liter of medium]

Item	7 days	17 days	28 days
	Milligrams	*Milligrams*	*Milligrams*
Total soluble material	17,852	18,120	16,220
Soluble ash	5,108	5,780	5,060
Minerals added to the medium [1]	1,850	1,770	1,680
Ash due to CaO	3,258	4,010	3,380
Lactic acid calculated from CaO	10,426	12,832	10,816
Sugar left in medium	3,263	0	0
Total soluble material accounted for [2]	17,886	17,466	14,910
Precipitation of soluble material with alcohol, total yield obtained	13,320	14,397	9,528
Total ash in precipitates	3,552	3,715	2,583
Ash calculated as Ca^{++}	2,573	2,653	1,845
Lactic acid by difference	10,747	11,744	7,683
	Percent	*Percent*	*Percent*
Ash found in lactate preparation [3]	26.7	25.8	27.1

[1] This is calculated from total minerals added minus the minerals in the ash in pellicle.
[2] Allowing for the CaO as Ca^{++} in the medium.
[3] Theoretical ash content in Ca lactate 25.7 percent; the slightly higher ash content found was later shown to be due to small amounts of phosphate impurities accompanying the lactate.

of the culture. After 7 days of growth of the organism, correlation between the two sets of results was very good. However, after 17 days, the yield of lactic acid by precipitation was somewhat less (8.5 percent) than that calculated from the ash content, and after 28 days the discrepancy was even greater (29.9 percent). Possibly this is due to the fact that, when all the sugar has disappeared, some of the lactic acid may become further oxidized to lower acids, as has been suggested previously (*7*, *11*, *29*).

INFLUENCE OF DIFFERENT FACTORS ON GROWTH OF RHIZOPUS AND PRODUCTION OF LACTIC ACID

REACTION OF THE MEDIUM

As pointed out above, the growth of *Rhizopus* upon carbohydrate media, as well as the formation and accumulation of lactic acid, is influenced considerably by the reaction and buffer content of the medium. In the following experiment the reaction was adjusted daily by the addition of sterile 0.5 N KOH solution. Some of the flasks were kept at pH 6.0, while others were made slightly alkaline, that is, pH 7.5 or higher. The results presented in table 5 show that when the reaction of the medium was kept alkaline, the organism produced more abundant growth but less lactic acid; in 12 days, there was more than twice as much growth at pH 7.5 as at pH 6.0, with about

half as much lactic acid produced. Thus conditions favorable to growth are unfavorable to lactic acid accumulation since more of the intermediary compound is used for energy, growth, and the metabolic activities of the organism. The nature of the acid formed was exactly the same when KOH was used as the neutralizing agent as when $CaCO_3$ was used.

TABLE 5.—*Influence of reaction of medium on growth of and lactic acid production by Rhizopus after 12 and 28 days*

[Results on the basis of 1 liter of medium]

Item	Medium pH 6.0		Medium pH 7.5+
	12 days	28 days	12 days
	Milligrams	*Milligrams*	*Milligrams*
NH_3N consumed	85	175	197
Fungus pellicle	1,680	2,670	3,790
N found in growth	61	142	178
Total soluble material	17,730	14,040	10,800
Soluble ash	7,540	5,640	5,040
Soluble K_2O	5,930	3,970	3,500
Lactic acid calculated from K_2O	11,360	7,600	6,700
Total soluble material accounted for	17,890	12,570	11,150
Lactic acid obtained by precipitation with alcohol as Ca salt	11,440	8,360	----------
Purified Ca $(C_3H_5O_3)_2$	9,600	----------	----------
	Percent	*Percent*	*Percent*
Ash in lactate	26.4	27.0	----------

In order to elucidate further the influence of reaction upon the metabolism of the organism, an experiment was made in which starch was used as the carbohydrate. In some of the flasks the reaction was adjusted daily to pH 5.5 and 6.5 by the use of sterile 0.25 N KOH solution; in the other flasks the reaction remained acid (about pH 4.0 or less). At the end of 4, 8, and 15 days, some of the flasks were removed for analysis. The organism began to grow rapidly at pH 4.0 (table 6), but in the absence of a neutralizing agent, the lactic acid

TABLE 6.—*Influence of reaction of medium on growth of and lactic acid production by Rhizopus in starch media after 4, 8, and 15 days*

[Results on the basis of 1 liter of medium]

Item	Medium pH, 4.0			Medium pH, 5.5			Medium pH, 6.5		
	4 days	8 days	15 days	4 days	8 days	15 days	4 days	8 days	15 days
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
Starch left as sugar	3,430	3,180	0	1,690	0	0	2,850	0	0
Sugar in medium	10,010	4,770	4,380	6,500	290	0	5,730	160	0
Carbohydrate consumed, as sugar	5,710	11,200	14,770	10,960	18,860	19,150	10,570	18,990	19,150
Growth of fungus	2,150	2,510	2,210	1,300	2,640	3,480	1,480	2,010	3,430
Nitrogen in growth	101	93	93	73	134	168	71	108	176
Lactic acid produced [1]	----------	4,320	3,300	5,890	12,730	10,750	7,300	13,070	10,390

[1] Calculated.

soon made conditions unfavorable for further growth, by lowering the pH, so that, even after 15 days, considerable carbohydrate was left in the medium. At the less acid reaction growth was at first slower and was accompanied by more abundant lactic acid production. The

carbohydrate nearly disappeared in 8 days, but the organism continued to grow, presumably at the expense of the intermediary substance.

Three distinct steps are thus observed in the transformation of starch by *Rhizopus*. (1) The hydrolysis of the polysaccharide to sugar. This process is independent of the reaction of the medium within the pH range used and it depends entirely on the growth of the fungus. (2) The transformation of the sugar to lactic acid. This process is markedly influenced by the reaction of the medium. At a high acidity, only a limited amount of acid is formed, but the organism continues, to decompose the sugar, without producing any acid. Whether, under these conditions, the acid is formed and is again oxidized, or whether the sugar is oxidized directly remains to be determined. (3) Possible oxidation of the lactic acid to CO_2 through the stage of certain lower organic acids. This process has been observed only in old cultures, hence possibly it is not to be considered in the system of glucose dissimilation by the organism. As mentioned above, small quantities of fumaric acid were constantly found in these cultures. In this respect the organism behaves similar to *Rhizopus oryzae* (*11*). In the second step, the possibility is not excluded that, in addition to lactic acid, sugar gives rise to another intermediary product which is gradually oxidized further, before the lactic acid is itself attacked.

TEMPERATURE

Lockwood et al. (*11*) found that *Rhizopus* grew better at 40° than at 30° C., but that the yield of lactic acid and especially the efficiency of its production, or the ratio of glucose consumed to acid produced, were less at the higher temperature. A series of liter flasks containing 500-cc portions of 5-percent potato starch were inoculated with spores of the fungus and incubated at 28° for 48 hours. Sterile $CaCO_3$ was then added to all the flasks and these were incubated for 9 days at different temperatures. The flasks were shaken daily by hand, for a few minutes. The results (table 7) show that although the optimum

TABLE 7.—*Influence of temperature on growth of and lactic acid production by Rhizopus on potato-starch medium at different temperatures*

[Results on the basis of 1 liter of medium containing 50 g of raw potato starch]

Temperature (° C.)	Sugar left as as glucose	Lactic acid produced [1]	Weight of fungus pellicle
	Milligrams	*Milligrams*	*Milligrams*
20	9,100	24,900	2,800
28	3,400	34,200	3,200
37	400	30,300	3,600
45–50	29,100	5,400	300

[1] Calculated.

temperature for growth was 37°, that for acid production was 28°. Above and below these temperatures there was a rapid reduction in growth, an increase in sugar production from starch, and a decrease in lactic acid formation. Conditions unfavorable for the production or accumulation of lactic acid are not necessarily unfavorable for the formation of sugars from starch, a finding which again suggests a

difference in the mechanisms responsible for the processes of starch hydrolysis and glycolysis of sugar.

In a further experiment pellicles were employed for the study of the influence of temperature on the production of lactic acid from glucose. The fungus was grown at 28° C. in the presence of $CaCO_3$, the filtrate was removed, and the pellicles were washed with sterile water. Sterile glucose solution and sterile $CaCO_3$ were added to the flasks containing the pellicles, and these were incubated at different temperatures for 2 and 4 days. The results obtained (table 8) were similar to those of the preceding experiment (table 7).

TABLE 8.—*Influence of temperature on lactic acid production by Rhizopus pellicle on glucose medium at different temperatures*

[Results on the basis of milligrams per liter of medium containing 25 g glucose]

Temperature of incubation (° C.)	2 days' incubation		4 days' incubation	
	Sugar consumed	Lactic acid produced	Sugar consumed	Lactic acid produced
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*
20	11,430		13,730	8,030
28	13,140	10,270	21,420	13,820
35	9,780	5,660	16,700	9,120
42	7,170	2,690	13,900	5,090
50	1,050	960	Trace	380

NITROGEN SOURCE

It has been shown (*29*) that nitrate is not a favorable source of nitrogen for the growth of *Rhizopus* or for lactic acid production, whereas ammonium salt is. Repeated comparisons between urea and ammonium sulphate as sources of nitrogen brought out the fact that both are favorable, provided precautions are taken not to split the urea to ammonium carbonate on sterilization, thus causing an alkaline reaction of the medium. In many of the subsequent experiments, urea was used as a source of nitrogen for the growth of the fungus, sterile urea solution being added to the medium. When fully grown pellicles were employed, the presence of available nitrogen was found to favor continued growth and to repress lactic acid accumulation.

RARE AND HEAVY METALS

The results of previous experiments brought out the fact that conditions favorable to the growth of the fungus are usually unfavorable to the production or accumulation of lactic acid. An attempt was made to find some chemical element or compound which would influence these two processes differently. The effect of salts of heavy metals and cell poisons was, therefore, studied. Records of a number of investigations are found in the literature concerning the action of heavy metals upon species of *Aspergillus*. According to McHargue and Calfee (*15*), the growth of *Rhizopus nigricans* is favored by the addition of Cu, Mn, and Zn, and especially by mixtures of these, in concentrations of 1 to 5 parts of the metal per million parts of medium. Lockwood et al. have also shown that zinc, in concentrations of 10 to 50 mg per liter, stimulates the growth of *Rhizopus* and the consumption of glucose; however, the presence of this element was found to result in a reduction in the yield of lactic acid

In the present studies, the basic medium consisted of 2 percent of glucose, 0.1 percent of $(NH_4)_2SO_4$, 0.05 percent of K_2HPO_4, and 0.05 percent of $MgSO_4.7H_2O$. The salts of the various elements tested were added on an anhydrous basis. Technical glucose was employed in the first experiment and c. p. anhydrous glucose in the others. In order that the effect of an organic source of nitrogen might be compared with that of the inorganic salt, 0.2 percent of peptone was substituted for the ammonium salt in one series of flasks. Some of the flasks received $CaCO_3$ in excess (20 g per liter) and the others were left free of carbonate. Analyses were made in duplicate after 7, 11, and 25 days' incubation at 28° C. and in a few cases after 4 days.

TABLE 9.—*Influence of heavy metals and certain rare elements, in the presence and in the absence of calcium carbonate, on the growth and acid production by Rhizopus at different stages of development*

[Results on the basis of 1 liter of medium]

Treatment	Growth in [1]—			Glucose consumed in—		Nitrogen consumed in—		Lactic acid produced in [2]—			
	7 days	11 days	25 days	4 days	7 days	7 days	11 days	4 days	7 days	11 days	25 days
Calcium carbonate used	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
Control	2,100	2,030	1,950	------	17,840	77	93	------	13,550	10,440	10,660
$FeCl_3$	1,580	1,570	2,180	4,420	17,080	55	60	5,390	14,140	13,920	8,400
$ZnSO_4$	4,520	3,620	3,750	13,170	18,360	185	176	5,890	7,750	5,770	3,600
$MnSO_4$	1,950	2,070	------	------	17,290	72	91	------	13,270	12,930	------
$CuSO_4$	2,150	------	------	1,560	16,180	79	------	Tr.	11,560	------	------
Na_3BO_3	2,050	2,150	------	------	17,630	70	102	------	15,930	10,700	------
Na_2MoO_4	2,250	2,150	2,070	------	17,790	82	97	------	12,740	10,790	9,890
Peptone [3]	2,030	1,990	2,000	------	17,950	81	[4] 91	------	15,160	11,720	12,250
No calcium carbonate											
Control	2,000	------	------	------	12,840	91	------	------	------	------	------
$FeCl_3$	1,480	------	------	5,910	9,690	85	------	5,330	------	------	------
$ZnSO_4$	3,500	------	2,790	10,810	14,480	185	------	2,570	------	------	5,670
$MnSO_4$	2,420	------	------	------	11,260	110	------	------	------	------	------
$CuSO_4$	2,490	------	------	4,320	11,010	117	------	3,470	------	------	------
Na_3BO_3	1,430	------	------	------	11,440	62	------	------	------	------	------
Na_2MoO_4	1,820	1,580	------	------	12,710	77	94	------	------	------	------
Peptone	1,950	1,530	1,730	------	12,480	[4] 74	[4] 68	------	------	------	3,350

[1] The pellicle in the $CaCO_3$ flasks was calculated from the nitrogen content, as found in the pellicles grown in the $CaCO_3$-free flasks, to avoid washing with HCl.
[2] Calculated from calcium in solution.
[3] In place of NH_4 as source of nitrogen.
[4] Nitrogen actually found in pellicle.

The results of the first experiment (table 9) show that the salts of various heavy metals and of other elements not commonly employed in culture media had a different effect on the growth and lactic acid production of *Rhizopus*. Some elements, such as iron and copper, had a depressing effect, in the concentrations used, both on growth and glucose consumption, but the effect on acid production was favorable. Other elements, especially zinc, were highly stimulating to the growth of the organism and to sugar consumption, but the effect on acid accumulation was depressive. Certain elements, such as manganese and molybdenum, had little additional effect on either growth or acid formation. Boron stimulated acid production at first, but later this effect tended to disappear. Peptone had no advantage over the ammonium salt as a source of nitrogen and con-

tributed none of the so-called biotic factors, for which this organism has no particular need. The results of this experiment point definitely to the presence of certain elements, notably zinc, as favoring growth of the fungus. Growth, however, takes place at the expense of the formation of lactic acid.

Further experiments were performed in which various concentrations of some of the above elements, as well as combinations of these, were used in order to determine whether both maximum growth and maximum acid production can take place simultaneously. Anhydrous c. p. glucose (2.5 percent) was used in this experiment; 0.2 percent of $(NH_4)_2SO_4$, 0.1 percent of K_2HPO_4, and 0.05 percent of $MgSO_4.7H_2O$ were added to make up the stock medium; the salts of the various elements were calculated on an anhydrous basis. All the flasks received $CaCO_3$, 2 days after inoculation.

TABLE 10.—*Influence of heavy metals, and certain rare elements in different concentrations on the growth and acid production by Rhizopus after 5, 11, and 18 days*

[Results on the basis of 1 liter of medium]

Treatment [1]	Growth in—			Glucose consumed	Nitrogen consumed in—			Lactic acid produced in—		
	5 days	11 days	18 days	5 [2] days	5 days	11 days	18 days	5 days	11 days	18 days
No Fe, no Zn	2,450	2,950	3,090	19,620	104	123	126	13,580	13,700	13,800
Fe:										
2 mg	2,420	3,380	3,160	18,340	97	129	129	12,960	12,960	13,390
5 mg	2,270	3,070	2,870	18,350	85	117	126	13,550	13,670	14,040
20 mg	1,270	2,310	2,530	14,070	41	87	117	12,400	15,280	14,070
Zn:										
5 mg	5,920	6,380	5,620	20,810	305	313	261	6,050	7,810	8,840
20 mg	5,000	6,820	------	20,490	258	337	------	7,560	5,860	------
5 mg Fe+5 mg Zn	6,970	7,020	7,400	20,880	361	370	379	2,760	4,340	3,160
5 mg Fe+10 mg Zn	6,200	8,020	7,420	19,460	332	379	379	3,940	4,030	5,020
5 mg Fe+5 mg Zn+5 mg Mn	6,700	8,330	8,180	20,630	358	379	379	2,910	3,660	4,090
5 mg Fe+5 mg Zn+5 mg Mn +5 mg Cu	6,800	8,030	8,140	21,100	370	379	379	2,640	3,350	2,670
Na_3BO_3, 5 mg	1,770	------	2,650	18,180	72	------	123	13,760	------	14,350
Na_3BO_3, 10 mg	2,010	------	2,650	18,410	92	------	117	12,960	------	13,890

[1] $Fe=Fe_2(SO_4)_3$, $Zn=ZnSO_4.7H_2O$, $Mn=MnSO_4.5H_2O$, $Cu=CuSO_4.5H_2O$, all on an anhydrous basis; milligrams of salt per liter of medium.

[2] All sugar (24,050 mg) consumed in 11 days.

Zinc was again found (table 10) to be specific in catalyzing the growth of the fungus. None of the other elements tested could take its place; they merely supplemented it by stimulating growth further. The effect of iron upon growth was, by itself, limited; however, when zinc was also added, there was an increased stimulating effect. The same was true of manganese and possibly copper. The influence of the last three elements was exerted directly upon the growth of the fungus (fig. 3). In all cases increased growth was accompanied by a reduction in the amount of lactic acid produced. This suggests the possibility that these elements favor oxidation of the precursor of lactic acid, thus resulting in a decrease in the formation of this acid, and thereby making more energy available for the growth of the fungus. In order to determine whether the organism is capable of oxidizing the lactic acid itself, an attempt was made to grow it on a calcium lactate medium, with and without the zinc salt. It was

found that neither spore inoculation nor young pellicles made any growth with lactic acid as the sole source of energy.

The following experiment illustrates further the specific effects of zinc on the growth and acid production of *Rhizopus*, as well as on the lactic acid formed. Eight liters of medium containing 5 percent of c. p. glucose and nutrient salts were divided into two lots, one receiving 0.001 percent of $Fe_2(SO_4)_3$ and the other 0.001 percent of $ZnSO_4$. The media were distributed, in 400-cc portions, into 1-liter flasks, sterilized, and inoculated. Ten-gram portions of $CaCO_3$ were added to some of the flasks. These were incubated at 28° C. and analyzed in duplicate after 6, 11, 16, and 25 days. The filtrates were evaporated to a small volume, precipitated with alcohol, and the lactate recovered.

The results presented in table 11 show that, although in the presence of zinc the sugar was consumed more rapidly, the growth of the fungus was even more rapid. After 6 days' incubation, the consumption of the sugar was, in favor of the zinc, 38.47: 26.05 or a ratio of 1.48:1, but, the corresponding dry weights of the fungus pellicle for the same period were 4.78:1. This extensive development of the fungus took

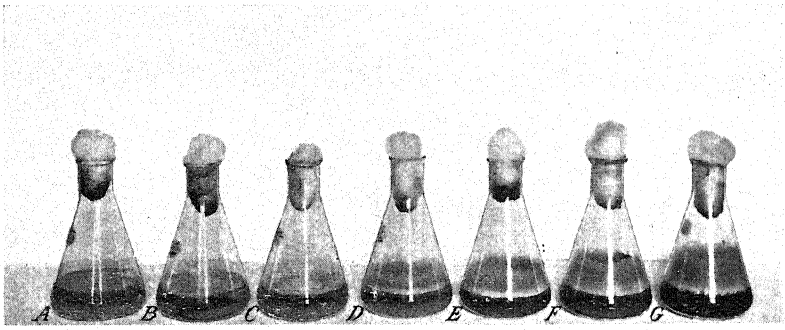


FIGURE 3.—Influence of different elements upon the growth of *Rhizopus*, on liter basis: *A*, Control: *B*, 2 mg $Fe_2(SO_4)_3$; *C*, 5 mg $Fe_2(SO_4)_3$; *D*, 5 mg $ZnSO_4$; *E*, 10 mg $Fe_2(SO_4)_3$ + 10 mg $ZnSO_4$; *F*, 5 mg each of $Fe_2(SO_4)_3$, $ZnSO_4$, and $MnSO_4$; *G*, 10 mg each of Fe, Zn, Mn, and Cu (as $CuSO_4$) salts.

place at the expense of the formation of lactic acid, which was reduced to less than one-half in the presence of zinc as compared with that in cultures not containing zinc. Similar results were obtained after 11, 16, and 22 days, when all the sugar had disappeared both in the presence and in the absence of zinc. The growth of the fungus was still twice as great in the presence of zinc as in its absence and the production of lactic acid was greatly reduced. The nitrogen was also consumed more rapidly in the cultures with zinc, so that additional ammonium salt had to be introduced (250-mg portions per flask), after 7 and 12 days' incubation.

The results of another experiment on the decomposition of glucose, in the presence of $CaCO_3$, are presented graphically in figure 4. In the absence of zinc, lactic acid was rapidly produced in the cultures of *Rhizopus* and was allowed to accumulate. At first the acid yield was high; then it gradually lessened up to about 13 days, when it remained constant. In the presence of zinc, the yield of lactic acid was considerably lower; however, when maximum growth was attained, lactic acid began to accumulate. It is of particular interest to note that, during

TABLE 11.—*Influence of iron and zinc upon the sugar and nitrogen consumed and the lactic acid produced by Rhizopus, and on its growth, in the presence and in the absence of calcium carbonate*

[Results on the basis of 1 liter of medium containing 5 percent of anhydrous glucose]

Period of incubation (days)	$CaCO_3$ added	Sugar consumed		Nitrogen consumed		Growth of fungus		Lactic acid calculated from CaO		Lactic acid actual yield	
		Fe	Zn	Fe	Zn	Fe	Zn	Fe	Zn	Fe	Zn
		Grams	*Grams*	*Milligrams*	*Milligrams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
6	+	26.05	38.47	97	336	1.38	6.60	15.45	7.43	11.50	7.06
11	+	42.00	46.20	170	486	3.83	9.03	21.81	8.55	18.26+	6.83+
16	+	46.20	46.20	224	495	5.08	10.36	24.21	9.83	16.00+	6.14+
22	+	46.20	46.20	233	494	5.57	11.78	22.64	9.16	17.98	6.61
11	−	15.07	13.64	139	236	2.29	3.33	Tr.	Tr.	------	------
22	−	19.57	18.87	192	239	2.64	3.01	Tr.	Tr.	------	------

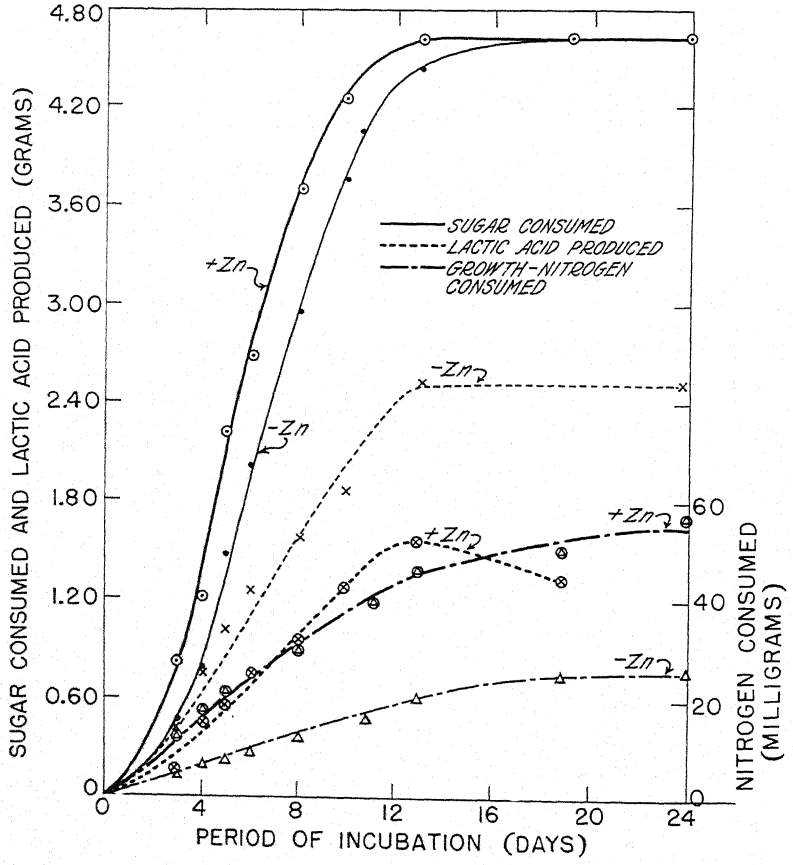


FIGURE 4.—Course of sugar decomposition, growth (nitrogen consumption), and lactic acid production by *Rhizopus*, as influenced by the presence of zinc in the medium.

the early stages of growth of the fungus, there was an inverse parallelism between growth and lactic acid production, both in the absence and in the presence of zinc. These results tend to show that zinc favors a more abundant growth of the organism, which is accompanied by a more rapid and complete destruction of the sugar, while lactic acid does not accumulate. In the absence of zinc, the carbohydrate is transformed largely to the lactic acid, with the liberation of a smaller amount of energy, as a result of which growth of the fungus is much more limited.

INFLUENCE OF OXYGEN TENSION ON LACTIC ACID PRODUCTION BY FUNGUS PELLICLES

To obtain further information concerning the process of lactic acid production by *Rhizopus*, it was necessary either to eliminate the growth of the organism or to reduce it as much as possible. This could be accomplished by the use of fully developed pellicles. The fungus was grown in 200-cc portions of medium placed in 500-cc Erlenmeyer flasks. The medium contained, in addition to the ordinary minerals, 2.5 percent of glucose and 0.001 percent of $ZnSO_4$, and to obtain more vigorous growth $CaCO_3$ was usually added also. After 6 to 7 days' incubation at 28° C., the filtrate was removed and the pellicle washed thoroughly with sterile distilled water. Sterile portions of sugar solution were then added to the flasks containing the pellicles.

TABLE 12.—*Influence of oxygen tension on lactic acid production by Rhizopus pellicles, in the presence and in the absence of calcium carbonate, after 4, 7, 10, and 15 days of growth*

[Results on the basis of 1 liter of medium]

Item	4 days				7 days				10 days			15 days	
	Aerobic		Anaerobic		Aerobic		Anaerobic		Aerobic	Anaerobic		Aerobic	Anaerobic
	$CaCO_3$+	$CaCO_3$−	$CaCO_3$+	$CaCO_3$−	$CaCO_3$+	$CaCO_3$−	$CaCO_3$+	$CaCO_3$−	$CaCO_3$−	$CaCO_3$+	$CaCO_3$−	$CaCO_3$−	$CaCO_3$−
	G.	*G.*	*G.*	*G.*	*G.*	*G.*	*G.*	*G.*	*G.*	*G.*	*G.*	*G.*	*G.*
Sugar consumed	16.73	13.85	17.62	15.00	25.00	17.35	23.23	20.46	18.65	25.00	23.40	22.61	22.64
Free lactic acid	.42	4.32	.64	3.94	.15	5.28	.29	6.66	7.01	.29	6.15	7.84	6.00
Lactic acid as calcium salt	8.91	.07	7.41	1.17	14.79	.29	11.71	.55	0	12.29	1.63	.48	1.06
Total lactic acid	9.33	4.39	8.05	5.11	14.94	5.57	12.00	7.21	7.01	12.58	7.78	8.32	7.06
	Per-cent	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*
Sugar converted to lactic acid	56	32	46	34	60	32	52	35	38	50	33	37	31

The results of the first experiment (table 12) show that, under aerobic conditions and in the presence of $CaCO_3$, the pellicle of the fungus converted 56 percent of the sugar to lactic acid in 4 days and 60 percent in 7 days. In the absence of $CaCO_3$ the rate of glycolysis was slower and the yield of acid less. The small amount of acid reported as the calcium salt when no $CaCO_3$ was added was due to some carbonate adhering to the pellicle which was not removed by the washing process. The rate of glycolysis, as measured by the disappearance of the sugar, was practically the same under anaerobic as

under aerobic conditions, but the rate of lactic acid production was less under anaerobic conditions, usually about 50 percent, in the presence of $CaCO_3$. When the CO_2 liberated under anaerobic conditions and in the absence of $CaCO_3$ was collected and measured, it was found to be approximately equivalent to the amount that would be expected from reaction (1), i. e., equimolecular concentrations of sugar consumed.

These results prove conclusively that lactic acid formation can take place under anaerobic conditions, by a fermentative mechanism. They prove further that the presence of oxygen does not hinder the production of lactic acid but may actually favor it, although the production process is accompanied by the consumption of some of the energy and carbon for growth purposes. These results suggest further that the enzyme system of the pellicle transforms the carbohydrate to lactic acid and to some other unidentified substance of the nature of an alcohol. This was later shown to be ethyl aclohol, by formation of the dinitro-benzoic acid ester. Under aerobic conditions this alcohol or its precursor is oxidized further, yielding energy for growth and giving more lactic acid.

The above experiment was repeated with pellicles grown for 8 days on a urea–$CaCO_3$ medium (containing also glucose, $ZnSO_4$ and other minerals). The pellicles were washed and sterile 2.5-percent glucose solution added. Some of the flasks were thoroughly aerated by bubbling air through the culture 5 to 8 hours every day, while others were kept in a CO_2 atmosphere. After 5 days' incubation at 28° C., all the flasks were removed for analysis. The results obtained (table 13) confirmed those of the previous experiment in showing that under anaerobic conditions and in the presence of $CaCO_3$, only 50 percent of the carbohydrate was converted to lactic acid, while in the presence of an excess of oxygen more lactic acid was formed.

TABLE 13.—*Influence of aeration on lactic acid production by Rhizopus pellicles in the presence and in the absence of calcium carbonate*

[Results on the basis of 1 liter of medium]

Item	Aerated		Anaerobic	
	$CaCO_3$	No $CaCO_3$	$CaCO_3$	No $CaCO_3$
	Grams	*Grams*	*Grams*	*Grams*
Sugar consumed [1]	26.70	26.70	23.56	24.37
Free lactic acid		7.00		7.81
Lactic acid as calcium salt	15.36	4.06	12.12	2.61
Total lactic acid	15.36	11.06	12.12	10.42
	Percent	*Percent*	*Percent*	*Percent*
Sugar converted to lactic acid	57.5	41.4	51.5	42.8

[1] Sugar in control was 26.70 g.

The pellicles were also found to be capable of converting starch to lactic acid. In the following experiment a sterile 2.5-percent solution of raw starch was added to 7-day-old pellicles grown on glucose + $CaCO_3$ medium. No nutrients were added to the starch solution. The flasks were incubated at 28° C. under anaerobic conditions for 7 days (table 14). All the starch disappeared (iodine test) and only a small amount of reducing sugar was left. The amount of starch trans-

formed to lactic acid was also about 50 percent, in the presence of $CaCO_3$. No volatile acid was formed except in mere traces as a result of the action of the pellicle on glucose and starch. However, large amounts of ethyl alcohol were demonstrated, especially in the anaerobic cultures. The formation of this alcohol accounts, together with the CO_2, for that part of the carbohydrate which was not converted to lactic acid.

TABLE 14.—*Lactic acid production from starch by pellicles of Rhizopus, in the presence and in the absence of calcium carbonate*

[Results on the basis of 1 liter of 2.5-percent starch solution [1]]

$CaCO_3$ added	Sugar left	Starch, as sugar, consumed	Free lactic acid	Combined lactic acid	Total lactic acid
	Grams	*Grams*	*Grams*	*Grams*	*Grams*
(−)	2.29	22.59	7.70	2.21	9.91
(+)	.87	24.01	0	13.12	13.12

[1] Total starch in 1 liter of medium equivalent to 24.88 g of reducing sugar.

The influence of nitrogen and minerals on glycolysis of sugar by the fungus pellicle is brought out in table 15. The data there shown emphasize the fact that the addition of available nitrogen favors the further growth of the pellicle and results in a depressing effect upon lactic acid production; however, the addition of minerals only, without the nitrogen, favored increased production of the acid.

TABLE 15.—*Influence of nitrogen and minerals on lactic acid production by Rhizopus pellicles* [1]

[Results on the basis of 1 liter of medium]

Item	Treatment		
	Urea [2]	Minerals [3]	Urea + minerals
	Grams	*Grams*	*Grams*
Sugar consumed	25.82	25.16	24.99
Lactic acid formed	11.23	17.66	10.13
	Percent	*Percent*	*Percent*
Sugar converted to acid	43.5	70.2	40.5

[1] Pellicles similar to those used in previous experiment (table 14) were employed; all flasks received $CaCO^3$; flasks kept stationary, with oxygen admitted.
[2] 0.1 percent.
[3] Equivalent to those added to medium.

By increasing the concentration of glucose, a single pellicle weighing approximately 1 g, on a dry basis, was found to be able to convert completely 40 g of glucose, in the presence of $CaCO_3$, to lactic acid and alcohol. In the presence of 20 percent of glucose, sufficient lactic acid was formed to solidify the medium because of the crystallization of the calcium lactate. The relation between glucose concentration and time of action of pellicle is brought out in figure 5. The proportional relationship definitely points to an enzyme mechanism. Concentrations of glucose above 20 percent had a depressing effect on glycolysis and resulted in the autolysis of the fungus cells.

The results of these and of other experiments, not reported here, lead to the following conclusions: (1) The presence of nitrogen and mineral nutrients is not necessary, in the case of fungus pellicles, for active lactic acid production to set in immediately; (2) the rate of formation of lactic acid by the pellicle depends on its age and vigor, rather than on the nature of the medium in which it is grown; (3) $CaCO_3$ is not necessary for the production of lactic acid by the pellicle, although more of the acid accumulates in the presence of the carbonate than in it absence; (4) there is no difference in the nature of the acid produced during the growth of the organism or that produced by the

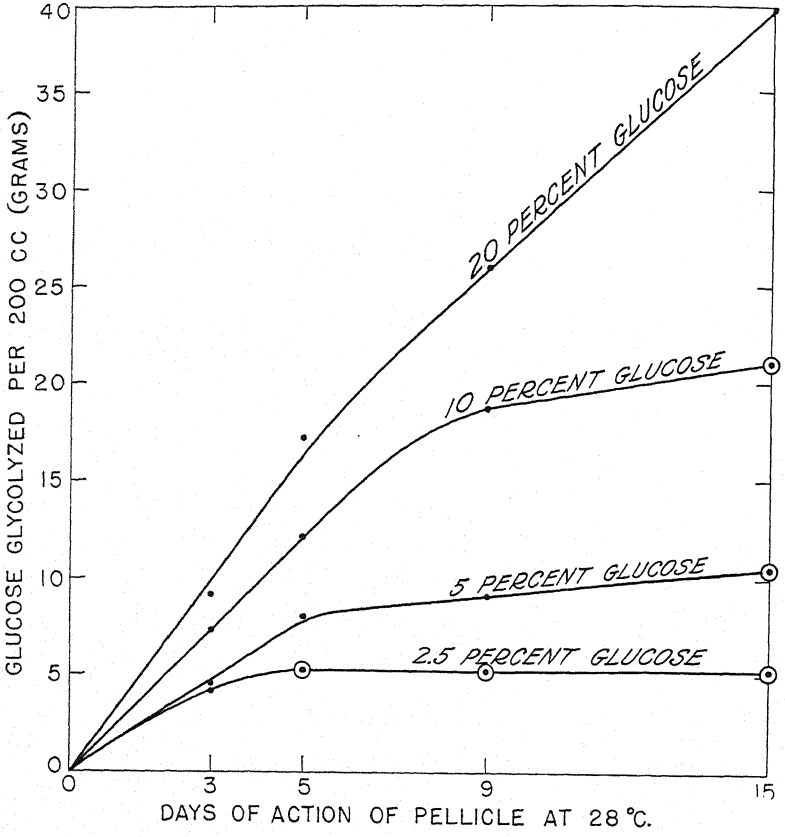


FIGURE 5.—Influence of glucose concentration on the rate of glycolysis by *Rhizopus* pellicles. Circles indicate that all the sugar has disappeared.

action of the pellicles on sugar or starch solution; (5) the amount of lactic acid produced by the pellicles acting on sugar depends to a certain extent on the oxygen tension, more lactic acid being formed under aerobic conditions; (6) under anaerobic conditions, abundant formation of ethyl alcohol takes place; (7) the fungus pellicle does not continue to grow under anaerobic conditions, whereas some growth occurs under aerobic conditions, especially when air is passed through the cultures and when additional nitrogen is present.

LACTIC ACID PRODUCTION FROM DIFFERENT CARBOHYDRATES AND RELATED COMPOUNDS BY RHIZOPUS PELLICLES

In all the previous experiments, glucose and starch were used as sources of carbon. In order to compare the production of lactic acid from other organic compounds by the fungus pellicle, a variety of sugars and sugar alcohols were selected. The organism was grown for 7 days upon a glucose solution containing the necessary nutrients and $CaCO_3$. The filtrate was removed, the pellicles were washed and the various compounds added, in concentrations of 2.0 to 2.5 percent, in the form of sterile solutions. All the flasks received $CaCO_3$ and were incubated at 28° C., at ordinary atmospheric pressure. The results, presented in table 16, show that active lactic acid formation by the fungus pellicle took place from glucose, maltose, dextrin, and levulose, and to a lesser extent from galactose and xylose. Although the organism could utilize lactose, rhamnose, and mannitol for growth, it produced no lactic acid from them. Attention may be called to the fact that in 1911 Saito (*27*) reported that *Rhizopus chinensis* produced lactic acid from glucose, levulose, maltose, and dextrin, but not from sucrose, inulin, or lactose.

TABLE 16.—*Lactic acid production by Rhizopus pellicles from different carbohydrates and related compounds after 3 and 7 days of growth*

[Results on the basis of 1 liter of medium]

Source of carbon	Reducing sugar consumed in—		Lactic acid produced—		Source of carbon	Reducing sugar consumed in—		Lactic acid produced—	
	3 days	7 days	3 days	7 days		3 days	7 days	3 days	7 days
	Grams	*Grams*	*Grams*	*Grams*		*Grams*	*Grams*	*Grams*	*Grams*
Glucose	14.35	19.62	5.56	8.45	Rhamnose		4.30		Tr.
Maltose	12.27	19.48	6.85	9.54	Lactose	0.4	2.51	0	Tr.
Dextrin	15.65	22.37	6.75	9.06	Sucrose [1]	0	0	0	Tr.
Arabinose	.14	3.54	Tr.	Tr.	Inulin	0	0	0	Tr.
Xylose	7.32	8.04	1.38	2.14	Mannitol [2]	1.48	3.13	0	Tr.
Galactose	10.91	11.12	2.62	4.54	Glycerol [2]	0	0	Tr.	Tr.
Levulose	14.45	22.55	9.15	10.21					

[1] On hydrolysis with dilute acid.
[2] Soluble material.

INFLUENCE OF POISONS ON GLYCOLYSIS BY RHIZOPUS PELLICLE

In order to determine the chemical nature of the break-down of the sugar by the fungus, a study was made of the influence of respiration poisons on the glycolytic effect of the pellicle. Substances known to repress the action of the glycolytic process, namely, sodium fluoride and iodoacetic acid, were utilized. Several 6-day-old pellicles were washed with water and 2.5-percent sterile glucose solution added to them. Different concentrations of the two poisons in sterile solutions were added. Some of the flasks received $CaCO_3$ and others did not. Incubation took place at 28° C. for 6 days in an anaerobic atmosphere. The results, presented in table 17, show that NaF, in concentrations of 0.02 to 0.10 percent, had only a limited effect on glycolysis and lactic acid production; these two processes were not repressed even with the highest concentrations of the poison. The iodoacetic acid repressed glycolysis in a concentration of 0.02 percent but not in the

lower concentrations used. The CO_2 liberated by the action of the pellicles was somewhat repressed by the poisons, especially in the higher concentrations. The larger amount of CO_2 produced in the presence of $CaCO_3$ was due to chemical interaction of the latter with the lactic acid.

A study was made of the influence of various concentrations of iodoacetic acid on the production of lactic acid by fully grown pellicles. These were obtained by growing the organism, for 6 days, upon a urea – $CaCO_3$–$ZnSO_4$ medium. The pellicles were washed with water and a 2.5-percent glucose solution was added. The flasks were incubated for 5 days at 28° C. in the presence of oxygen. Because of the short incubation period, the recovery of the acid was not complete. The results of this experiment (table 18) show again that increasing concentrations of the iodoacetic acid repressed both sugar glycolysis and lactic acid production, the latter to a much greater extent than the former. The pellicle glycolyzed the sugar even in the presence of 0.1 percent of the poison, but under similar conditions less than 10 percent of the sugar was recovered as lactic acid. This was true both when the reaction was neutral and when it was acid.

TABLE 17.—*Influence of different concentrations of glycolytic poisons on lactic acid production by Rhizopus pellicles in the presence and in the absence of calcium carbonate*

[Results on the basis of 1 liter of sugar solution]

Poison used	Concentration of poison	$CaCO_3$	Sugar consumed	Lactic acid produced	CO_2 produced (as C) [1]
	Percent		*Grams*	*Grams*	*Milligrams*
None	0.00	−	17.73	8.83	346
	.00	+	20.92	9.79	617
Sodium fluoride	.02	−	8.76	2.83	337
	.02	+	16.13	4.80	463
	.04	−	6.86	2.93	246
	.04	+	15.42	5.38	309
	.10	−	5.16	2.40	215
	.10	+	16.67	8.35	321
Iodoacetic acid	.004	−	9.00	2.45	375
	.004	+	11.94	6.82	552
	.008	−	8.84	4.01	305
	.008	+	11.79	5.67	374
	.02	−	4.11	.77	113
	.02	+	7.74	2.11	247

[1] The pellicle alone gave off, in an aqueous solution, 192 mg of carbon as CO_2, in the absence of $CaCO_3$, and 156 mg of carbon in the presence of $CaCO_3$.

TABLE 18.—*Influence of different concentrations of iodoacetic acid on sugar consumed and lactic acid produced by Rhizopus pellicles in the presence and in the absence of calcium carbonate*

[Results on the basis of 1 liter of sugar solution]

Concentration of iodoacetic acid (percent)	$CaCO_3$	Sugar consumed	Lactic acid produced	Sugar converted to lactic acid	Concentration of iodoacetic acid (percent)	$CaCO_3$	Sugar consumed	Lactic acid produced	Sugar converted to lactic acid
		Grams	*Grams*	*Percent*			*Grams*	*Grams*	*Percent*
0.00	+	23.4	9.2	39.3	0.00	−	22.6	5.9	26.1
.01	+	19.0	5.2	27.4	.01	−	17.8	4.3	24.2
.02	+	15.1	2.8	18.5	.02	−	13.7	2.6	19.0
.04	+	13.1	1.8	13.7	.04	−	12.7	1.2	9.4
.10	+	10.6	.9	8.5	.10	−	10.5	.7	6.7

An experiment was made to determine the effect of iodoacetic acid on the growth of the fungus. The regular ammonium sulphate-glucose-$ZnSO_4$ medium containing the different concentrations of the poison was inoculated with spores of the fungus and incubated for 6 days. Calcium carbonate was added only to those flasks that showed active growth of the fungus. The results (table 19) show that concentrations of iodoacetic acid greater than 0.01 percent in the medium completely represses the growth of *Rhizopus*.

TABLE 19.—*Influence of different concentrations of iodoacetic acid upon the growth of Rhizopus from spores*

[Results on the basis of 1 liter of medium]

Concentration of iodoacetic acid (percent)	Sugar consumed	Lactic acid produced	Concentration of iodoacetic acid (percent)	Sugar consumed	Lactic acid produced
	Grams	*Grams*		*Grams*	*Grams*
0.00	25.8	9.90	0.03	0.9	0.2
.01	24.3	9.3	.10	.3	0

FORMATION OF INTERMEDIARY PRODUCTS IN THE GROWTH OF RHIZOPUS

The results of the foregoing experiments have established definitely that nutrition and energy utilization of *Rhizopus* do not take place through the lactic acid stage, as originally assumed. Actually the lactic acid is a byproduct of the reaction and can be oxidized further by the organism only to a very limited extent, if at all. It is to the other product of the break-down of the sugar that one must look for the energy and carbon source of the organism. This product is ethyl alcohol; it is formed in the anaerobic break-down of the sugar; under aerobic conditions, it is either not formed at all or further oxidized. It may, of course, be assumed that the alcohol arises through the usual pyruvic acid stage; however, it is the alcohol which is the intermediary between the anaerobic and the aerobic phases of the respiration process. In other words, we have here a good example of the Pasteur reaction. This is clearly illustrated by the results of an experiment on the formation of alcohol by *Rhizopus* pellicles under aerobic and anaerobic conditions (table 20). Under anaerobic conditions the concentrations of lactic acid and of ethyl alcohol produced were practically equimolecular with the glucose consumed. Under aerobic conditions, however, the amount of alcohol found was very small and the lactic acid produced was much greater than the equimolecular concentration. Whether the excess lactic acid is produced from the alcohol or from its precursor still remains to be determined. The fact is that the organism is able to utilize ethyl alcohol readily as a source of energy and carbon.

TABLE 20.—*Glucose consumed and lactic acid and ethyl alcohol produced by Rhizopus pellicle under aerobic and anaerobic conditions*

[Results on the basis of 1 liter of medium]

Period elapsed (days)	Aeration	Glucose consumed		Lactic acid produced		Ethyl alcohol produced	
		Grams	*Millimols*	*Grams*	*Millimols*	*Grams*	*Millimols*
15	Anaerobic	53.9	300	28.5	316	13.1	286
20	do	57.3	318	29.4	327	15.8	344
15	Aerobic	82.8	460	61.5	684	4.3	94

DISCUSSION

Three distinct factors or groups of factors are primarily concerned in the growth of micro-organisms upon natural and artificial substrates, and to a large extent they control the formation and accumulation of intermediary and waste products of microbial metabolism. These factors are (1) biological, including the nature of the organism, its strain specificity, and its previous cultivation; (2) nutritional, comprising the nature of the energy source and its concentration, the nature and concentration of the nitrogen source, the nature and abundance of other nutritive elements, and the presence of certain special elements that have a positively or a negatively stimulating effect on the growth of the particular organism and on its specific metabolism; (3) environmental, including temperature, reaction, oxygen tension, length of cultivation, etc.

In the study of the physiology of the lower fungi, these factors were found not only to influence the quantitative accumulation of certain metabolic products but to modify the very nature of these products. It is sufficient to call attention to the preferential formation of gluconic acid or of citric and oxalic acids by *Aspergillus niger;* of ethyl alcohol or of glycerol by yeasts; of butyl alcohol or of butyric acid by certain anaerobic bacteria; of lactic acid or of acetic acid by acetic acid bacteria, etc. The results of the investigations reported in this paper on the mechanism and conditions of formation of lactic acid by a species of *Rhizopus* can also be interpreted in terms of the various nutritional and environmental factors.

Molliard (*19*, *20*, *21*) has shown that, in the presence of all the necessary nutrient salts, *Aspergillus niger* develops an extensive mycelium without allowing the accumulation of acids; acids accumulate only when certain essential nutritive elements are lacking. Butkewitsch (*2*) suggested that the specific formation of acids by *A. niger* depends on the reaction of the medium. Kuznetsov (*9*, *10*) attempted to establish a correlation between acid formation and the oxidation-reduction potential of the medium. Kanel (*7*) suggested that the formation of lactic acid by Mucorales, but not by other fungi, is due to the ability of the Mucorales to grow immersed in the liquid medium. He found that a change in the biochemical activities of the organism or its physiological polymorphism could be correlated with the specific nature of the organism and the oxidation-reduction potential of the medium. Kanel further attempted to differentiate between the fermentative and the oxidative phases of the fungus. The fact that aeration of the culture did not change the lactic acid yield led him to suggest that air acts only mechanically by mixing the liquid,

and since different oxidation-reduction potentials had no effect on the lactic acid yield, he believed that immersed *Rhizopus* growth is independent of the oxidation potential of the medium. A low potential repressed the formation of fumaric acid, however, which he considered to be a secondary oxidation product.

Plant physiologists (*24*) have long recognized two distinct phases in the process of energy utilization by cells of higher plants, namely, the fermentation phase, which comprises the production of lactic acid or of alcohol and CO_2, and the respiration phase, which results in the production of CO_2 and water. In the case of micro-organisms, lactic acid has usually been considered the final product of the anaerobic or fermentation reactions of certain specific bacteria. In discussing the metabolism of lactic acid bacteria, Kluyver (*8*) remarks that it is possible to have oxygen take part in the process; he adds, however, that "if respiration is defined as an oxygen consuming process which yields the energy necessary for the maintenance and proliferation of cells then the conclusion must be that lactic acid bacteria do not respire."

According to Neuberg (*22*), methylglyoxal forms the intermediary stage in the break-down of the sugar molecule to lactic acid. Meyerhof and Kiessling (*16*, *17*, *18*) and Embden et al. (*5*) do not ascribe to methylglyoxal any part in the process; they believe that phosphoglyceric and pyruvic acids form the important intermediary products. Lundsgaard (*12*, *13*, *14*) noted that the presence of sodium-monoiodoacetate, in certain concentrations, inhibits fermentation by yeast or by zymase, but has no effect upon oxygen consumption. He concluded therefore that sugar break-down in fermentations has no relation to its break-down in respiration. Some investigators believe that iodoacetic acid acts on the initial stages of glucose fermentation by inhibiting the phosphorylating mechanism, for in yeast (unlike muscle) no glucose disappears and no intermediate products are formed in the presence of iodoacetate (*4*).

In the experiments reported here, both lactic acid production and sugar break-down were repressed by iodoacetic acid, from which it may be assumed that lactic acid is a direct product of glycolysis. Other facts presented in this paper substantiate this assumption. The fact that a lower yield of lactic acid was obtained per unit of glucose consumed, both during growth and as a result of pellicle action, indicates direct oxidation of the glucose or of some of the precursors of lactic acid, even in the presence of the poison.

The following reactions are suggested to explain the mechanism of sugar break-down and respiratory process of *Rhizopus:*

$$C_6H_{12}O_6 \longrightarrow \underset{\text{(intermediate compound)}}{C_3H_6O_3} + \underset{\text{(intermediate compound)}}{C_3H_6O_3} \quad \text{(anaerobic)}$$

$$\underset{\text{(intermediate compound)}}{C_3H_6O_3} \longrightarrow \underset{\text{(lactic acid)}}{C_3H_6O_3} \quad \text{(anaerobic)}$$

$$C_3H_6O_3 \longrightarrow C_2H_5OH + CO_2 \quad \text{(anaerobic)}$$
(intermediate compound)

$$C_2H_5OH + 3O_2 \longrightarrow 2CO_2 + 3H_2O \quad \text{(aerobic)}$$

$$C_3H_6O_3 + 3O_2 \longrightarrow 3CO_2 + 3H_2O \quad \text{(aerobic)}$$

There are cases on record where lactic acid has been found to be an intermediary product in the metabolism of micro-organisms. Peterson and Fred (*25*) for example, have shown, that certain bacteria produce acetic acid through the lactic acid stage, by an oxidation process. The possibility that fumaric acid may be formed through this stage has also been indicated (*11*). However, the fungus under study could not initiate growth with lactic acid as the only source of energy.

The function of zinc in the metabolism of *Rhizopus* is of special interest. It has been recognized since the earliest works on the nutrition of fungi that these organisms require zinc, and this finding was recently confirmed for *Aspergillus niger* by Steinberg (*28*), Roberg (*26*), and others. However, considerable disagreement still exists concerning the role of this element in the metabolism of fungi. Some investigators have been satisfied to relegate the specific function of this element to that of a stimulant or biocatalyzer, whereas others have insisted that it is a normal nutrient, required in small amounts for the metabolism of the organism. Very little attention has been paid, in most of these investigations, to the role of zinc in the mechanism of transformation of the carbohydrate used by the organism as a source of energy. Ōno (*23*) suggested that *Aspergillus* utilizes the carbohydrate more economically in the presence of $ZnSO_4$, the intermediary product, oxalic acid being oxidized more rapidly. Similar results were obtained by Butkewitsch and Orlow, (*3*) who found that zinc repressed acid formation, oxalic acid disappearing almost completely in its presence. Javillier (*6*) concluded that the role of zinc in the growth of *A. niger* is that of a catalyst rather than of a nutrient; this action was specific and could not be replaced by any other element. Wassiljew (*30*) suggested that zinc influences primarily the metabolism of the fungus, notably its acid production; that it is an essential element for growth and not a stimulant; and that both vegetative growth of the fungus and the formation and consumption of the acids from sugar are influenced by the presence of this element. The influence of zinc differs, however, with different strains of *A. niger*.

According to Roberg (*26*), zinc is essential to the growth of Aspergilli; however, in solutions that do not contain sufficient iron, it produces an injurious effect. An antagonistic relation was believed to exist between the two elements. A similar relation was observed by McHargue and Calfee (*15*) in studies of the effect of copper, manganese, and zinc on growth of fungi. Buromskiĭ (*1*) emphasized recently that zinc must still be looked upon as a stimulant for the growth of fungi; he went so far as to claim that even potassium and phosphorus should be placed in this category. Zinc was also found to increase the respiration coefficient of fungi, that is, the ratio of CO_2

to growth, and to modify the nature of the acids produced (*19, 20, 21, 30*). In the case of *Aspergillus niger*, this was correlated with a delay in spore formation resulting in increased dry weight and extension of the cycle of growth of the organism.

In the present studies, zinc was shown to stimulate both growth and spore formation of *Rhizopus*, but not to favor acid production. These results indicate that zinc either catalyzes the oxidation of glucose or of the precursor of lactic acid directly to CO_2, thereby increasing the liberation of energy and stimulating growth. One need not argue, at this time, whether the elements zinc and iron make conditions for the growth of *Rhizopus* "normal" or "abnormal." The very fact that the fungus was grown on an artificial substrate with a relatively high concentration of carbohydrate, with inorganic sources of nitrogen and minerals, makes conditions of growth sufficiently "abnormal," as compared with those under which the organism is commonly found in nature, that is, on stale bread or in soil. One is, therefore, hardly justified in concluding that because zinc stimulates the growth of the fungus, it makes conditions "normal," while iron, which alone is not favorable to growth but is favorable to lactic acid production, makes conditions "abnormal."

SUMMARY AND CONCLUSIONS

This paper presents the results of a study of the nutrition of a fungus belonging to the genus *Rhizopus*, its ability to produce lactic acid from different carbohydrates, the mechanism of production of the acid, and the role of this process in the metabolism of the fungus.

In general, conditions favoring the growth of *Rhizopus* were found to be unfavorable to the accumulation of lactic acid. The fully grown pellicle placed in a sugar solution produced lactic acid to the extent of 50 percent of the sugar under anerobic conditions and of about 60 percent under aerobic conditions; in the latter case some growth accompanied the process of acid formation. Only about 30 to 40 percent of the carbohydrate consumed was changed to acid in the absence of a neutralizing agent, in growth cultures, and as much as 70 to 75 percent in the presence of such an agent.

One may conclude from these results that *Rhizopus* produces lactic acid by a fermentation mechanism despite it being a strictly aerobic organism. The lactic acid is preceded by an intermediary substance, which is converted by pellicles, under anaerobic conditions, to the extent of 50 percent to the acid and the remaining 50 percent to alcohol and carbon dioxide. Only a little energy is liberated in this reaction. During growth of the organism, or under aerobic conditions, the precursors of lactic acid and of alcohol are oxidized partly to lactic acid and partly to carbon dioxide. The presence of different elements influences the predominance of one reaction over another. Zinc favors growth and is thereby unfavorable to lactic acid production; iron, on the other hand, is highly favorable to lactic acid production and is not favorable to cell growth.

The fungus *Rhizopus* is shown to possess both a fermentative and an oxidative mechanism, similar to those of higher forms of life.

The essential features of this paper were presented at the meetings of the Society of American Bacteriologists, December 27–29, 1937, Washington, D. C.

(Jour. Bact. *35*, 70, 1938). After this paper was accepted for publication, a discussion by Ward et al. (Ind. Eng. Chem. *30*, 1233–5, 1938) concerning certain phases of the intermediary metabolism and the role of ethyl alcohol in the respiration of a lactic acid producing *Rhizopus* appeared, in which the findings presented in this paper were confirmed.

LITERATURE CITED

(1) BUROMSKIĬ, I. D.
1936. THE INFLUENCE OF ZN, MG, AND K ON THE METABOLISM OF ASPERGILLUS NIGER. Microbiology 5: 800–811. [In Russian. English summary.]

(2) BUTKEWITSCH, WL.
1924. ÜBER DIE BILDUNG DER GLUCON- UND CITRONSÄURE IN DEN PILZKULTUREN AUF ZUCKER. Biochem. Ztschr. 154: 177–190.

(3) ——— and ORLOW, FR. W. G.
1922. ZUR FRAGE NACH DEN "ÖKONOMISCHEN KOEFFIZIENTEN" BEI ASPERGILLUS NIGER. Biochem. Ztschr. 132: 556–565, illus.

(4) DIXON, M.
1937. ACTION OF INDOACETATE ON DEHYDROGENASES AND ALCOHOLIC FERMENTATION. Nature [London] 140: 806.

(5) EMBDEN, G., DEUTICKE, H. J., and KRAFT, GERT.
1933. ÜBER DIE INTERMEDIÄREN VORGÄNGE BEI DER GLYKOLYSE IN DER MUSKULATUR. Klin. Wchnschr. 12: 213–215.

(6) JAVILLIER, M.
1913. RECHERCHES SUR LA SUBSTITUTION AU ZINC DE DIVERS ÉLÉMENTS CHIMIQUES POUR LA CULTURE DE L'ASPERGILLUS NIGER (STERIGMATOCYSTIS NIGRA V. TGH.). ÉTUDE PARTICULIÈRE DU CADMIUM ET DU GLUCINUM. Ann. Inst. Pasteur 28: 1021–1038.

(7) KANEL, E.
1934. LACTIC ACID FORMATION IN THE CULTURE OF A FUNGUS OF THE RHIZOPUS SPECIES. Microbiology 3: 259–265. [In Russian. English summary, p. 265.]

(8) KLUYVER, A. J.
1933. SOME REMARKS ON THE METABOLISM OF THE LACTIC ACID BACTERIA AND ON THEIR RÔLE IN BUTTER MAKING. Jour. Soc. Chem. Indus. Trans. 52: 367T–373T.

(9) KUZNETSOV, S. I. (KUSNETZOW, S. J.)
1931. THE DEPENDENCE OF THE OXIDIZING FERMENTATIONS FROM THE OXIDATION-REDUCTION POTENTIAL OF THE EXTERNAL MEDIUM. Zentbl. Bakt. [etc.] (II) 83: 37–52, illus.

(10) ———
1932. CHANGES IN OXIDATION-REDUCTION PROCESSES OF ASPERGILLUS NIGER IN CONNECTION WITH CHANGES OF THE POTENTIAL IN THE MEDIUM. Microbiology 1: 30–48. [In Russian.]

(11) LOCKWOOD, L. B., WARD, G. E., and MAY, O. E.
1936. THE PHYSIOLOGY OF RHIZOPUS ORYZAE. Jour. Agr. Research 53: 849–857

(12) LUNDSGAARD, EINAR.
1930. DIE MONOJODESÄUREWIRKUNG AUF DIE ENZYMATISCHE KOHLENHYDRATSPALTUNG. Biochem. Ztschr. 220: 1–7, illus.

(13) ———
1930. ÜBER DIE EINWIRKUNG DER MONOJODESSIGSÄURE AUF DEN SPALTUNGS- UND OXYDATIONSSTOFFWECHSEL. Biochem. Ztschr. 220: 8–18, illus.

(14) ———
1932. WEITERE UNTERSUCHUNGEN ÜBER DIE EINWIRKUNG DER HALOGENESSIGSÄUREN AUF DEN SPALTUNGS- UND OXYDATIONSSTOFFWECHSEL. Biochem. Ztschr. 250: 61–88, illus.

(15) MCHARGUE, J. S., and CALFEE, R. K.
1931. EFFECT OF MANGANESE, COPPER, AND ZINC ON GROWTH AND METABOLISM OF ASPERGILLUS FLAVUS AND RHIZOPUS NIGRICANS. Bot. Gaz. 91: 183–193, illus.

(16) MEYERHOF, OTTO.
1936. MECHANISM OF THE ENZYMIC BREAKDOWN OF CARBOHYDRATES (FORMATION OF LACTIC ACID AND ALCOHOLIC FERMENTATION). Current Sci. 4: 669–682. [Abstract in Chem. Abs. 30: 5246. 1936.]

(17) MEYERHOF OTTO, and KIESSLING, W.
1933. ÜBER DAS AUFTRETEN UND DEN UMSATZ DER α-, GLYCERIN-PHOSPHORSÄURE BEI DER ENZYMATISCHEN KOHLENHYDRATSPALTUNG. Biochem. Ztschr. 264: 40–71, illus.

(18) ——— and KIESSLING, W.
1935. THE PRINCIPAL PATH OF LACTIC ACID FORMATION IN MUSCLES. Biochem. Ztschr. 283: 83–113. [Abstract in Chem. Abs. 30: 1857–1858. 1936.]

(19) MOLLIARD, MARIN.
1919. PRODUCTION D'ACIDE CITRIQUE PAR LE STERIGMATOCYSTIS NIGRA. Compt. Rend. Acad. Sci. [Paris] 168: 360–363.

(20) ———
1922. SUR UNE NOUVELLE FERMENTATION ACIDE PRODUITE PAR LE STERIGMATOCYSTIS NIGRA. Compt. Rend. Acad. Sci. [Paris] 174: 881–883.

(21) ———
1924. NOUVELLES RECHERCHES SUR LA FORMATION D'ACIDES ORGANIQUES PAR LE STERIGMATOCYSTIS EN MILIEUX DÉSÉQUILIBRÉS. Compt. Rend. Acad. Sci. [Paris] 178: 41–45.

(22) NEUBERG, C., and GORR, G.
1925. ÜBER DEN MECHANISMUS DER MILCHSÄUREBILDUNG BEI BAKTERIEN. Biochem. Ztschr. 162: [491]–495.

(23) OÑO, N.
1900. UEBER DIE WACHSTUMBESCHLEUNIGUNG EINIGER ALGEN UND PILZE DURCH CHEMISCHE REIZE. Jour. Col. Sci., Imp. Univ. Tokyo 13: 141–186, illus.

(24) PALLADIN, W.
1909. ÜBER DAS WESEN DER PFLANZENATMUNG. Biochem. Ztschr. 18: 151–206, illus.

(25) PETERSON, W. H., and FRED, E. B., with the cooperation of ANDERSON, J. A.
1920. THE FERMENTATION OF GLUCOSE, GALACTOSE AND MANNOSE BY LACTOBACILLUS PENTACETICUS, N. SP. Jour. Biol. Chem. 42: 273–287.

(26) ROBERG, MAX.
1928. ÜBER DIE WIRKUNG VON EISEN-, ZINK-, UND KUPFERSALZEN AUF ASPERGILLEN. Centbl. Bakt. [etc.] (II) 74: 333–370, illus.

(27) SAITO, K.
1911. EIN BEISPIEL VON MILCHSÄUREBILDUNG DURCH SCHIMMELPILZE. Centbl. Bakt. [etc.] (II) 29: 289–290.

(28) STEINBERG, ROBERT AARON.
1919. A STUDY OF SOME FACTORS IN THE CHEMICAL STIMULATION OF THE GROWTH OF ASPERGILLUS NIGER. Amer. Jour. Bot. 6: 330–372.

(29) WAKSMAN, SELMAN A., and HUTCHINGS, I. J.
1937. LACTIC ACID PRODUCTION BY SPECIES OF RHIZOPUS. Jour. Amer. Chem. Soc. 59: 545–547.

(30) WASSILJEW, G. M.
1935. ÜBER DIE EINWIRKUNG VON ZINK AUF DEN STOFFWECHSEL VON ASPERGILLUS NIGER. Arch. Mikrobiol. 6: 250–275.

DETERMINATION AND OCCURRENCE OF BORON IN NATURAL PHOSPHATES, SUPERPHOSPHATES, AND DEFLUORINATED PHOSPHATE ROCKS[1]

By LEWIS F. RADER, Jr., *assistant chemist*, and W. L. HILL, *associate chemist*, *Fertilizer Research Division, Bureau of Chemistry and Soils, United States Department of Agriculture*[2]

INTRODUCTION

Boron belongs to the gradually lengthening list of elements that are regarded as essential to plant growth. Recent papers review previous work on the effect of boron on plants (*1, 4, 8, 12, 26*)[3] and its occurrence in plants (*8*), soils (*8, 26*), and fertilizers including fertilizer materials (*5, 10, 11, 26*). The data for fertilizers and fertilizer materials, however, include only a few scattered results for boron in natural phosphates and superphosphates.

Results are given in this paper for boron in 54 representative samples of natural phosphates from various parts of the world, 9 samples of commercial superphosphates, and 3 samples of defluorinated phosphate rock. The results for boron in natural phosphates reported herein represent an extension of the studies of this Bureau on the composition of phosphate rock (*15, 18, 19*).

METHOD OF ANALYSIS

Chemical methods for determining boron have been classified and reviewed by Wilcox (*24*). The methods most commonly used for determining boron in minerals and fertilizers are the Chapin method (*23, p. 1691*), or some modification of it, and the official methods (*3, p. 32*). According to the Chapin method, boron is separated from the other constituents of the sample (fused with sodium carbonate, if insoluble in acid) by distillation with methyl alcohol in the presence of hydrochloric acid and of anhydrous calcium chloride as a drying agent. The methyl ester of boric acid formed in the absence of free water passes into the distillate, from which the boron is recovered in a small volume of water by saponification with alkali with subsequent removal of the alcohol by distillation. The alkaline aqueous solution of borate thus obtained is freed from carbonate and adjusted to a definite pH (6–7) after which the quantity of boron in the solution is determined by titration with standard alkali in the presence of mannitol to the pink color of the phenolphthalein indicator. In the official method for acid-soluble boron in fertilizers phosphoric acid, instead of hydrochloric acid, is used, and since phosphoric acid acts as a drying agent, this change renders the use of calcium chloride unnecessary.

The method used by the authors is substantially the method of Chapin, though the titration is in principle that of Foote (*9*). Diffi-

[1] Received for publication Sept. 14, 1938. Issued February 1939.

[2] The authors are indebted to K. D. Jacob, Fertilizer Research Division, who suggested the investigation and rendered valuable aid during the course of the work, and to J. I. Hoffman, National Bureau of Standards, for helpful suggestions on the application of the methods.

[3] Reference is made by number (italic) to Literature Cited, p. 914.

culties not met in the analysis of most materials heretofore studied are at once encountered in the application of the method to natural phosphates. Not only are the involved quantities of boron so small as to require reagents and apparatus that are sufficiently low in boron to reduce the blank correction to a very low value, but as a consequence of the presence of fluorine and silica in most natural phosphates the distillates contain relatively large quantities of hydrofluosilicic acid, which titrates in the same pH range (*17*) as does the complex of boric acid with mannitol. The authors' application of published methods to the determination of boron in fluorine-bearing phosphates and their observations on means of obviating the foregoing difficulties can be conveniently presented under the subjects: (1) Reagents, (2) titration of boric acid, (3) separation of boron from the sample, and (4) procedure used.

REAGENTS

Since the presence of fluorine in natural phosphates renders the glassware used in the course of their analysis a reagent in a very real sense, the use of borosilicate glassware is not permissible. Concentrated solutions of sodium hydroxide also become contaminated with boron from the glassware (*24*). Accordingly, Kavalier glassware (usually considered as boron-free) was used for the analytical operations and for the storage of alkaline reagent solutions. The boron content of different lots of the several reagent chemicals is shown in table 1. It may be pointed out that a 6 *M* solution of sodium hydroxide prepared from a more concentrated solution that remained in a Pyrex flask for several weeks contained far more boron (92 parts per million of B_2O_3) than did similar solutions prepared in Kavalier glass throughout (table 1).

TABLE 1.—*Boron in analytical reagents*

Reagent	B_2O_3 in reagent [1]		Reagent	B_2O_3 in reagent	
	As received	As used		As received	As used
	P. p. m.	*P. p. m.*		*P. p. m.*	*P. p. m.*
Sodium hydroxide	4	[2] 1	Calcium chloride, anhydrous for drying	53	2.4
Do	35	[2] 11	Methyl alcohol, absolute	4.5	0
Potassium hydroxide	7	[3]	Sodium carbonate	<5	<5
Do	66	[3]	Do	<5	<5
Calcium chloride, anhydrous for drying	32	.6	Mannitol	[4] 0	[4] 0

[1] Results include boron present in the hydrochloric acid used for neutralization.
[2] Milligrams of B_2O_3 per liter of 6 *M* solution.
[3] Not used.
[4] Result shown by blank titration on 2.5 g of mannitol.

A stock solution of carbonate-free sodium hydroxide (*13, p. 139*) was prepared by rinsing the sticks with distilled water to remove any surface boron arising from attack of the glass container, dissolving the rinsed sticks in an equal weight of distilled water, and allowing the solution to stand until the supernatant liquid was clear. Dilute solutions of carbonate-free sodium hydroxide were prepared as needed by diluting aliquots of this concentrated solution with recently boiled distilled water.

The reagent grades of anhydrous calcium chloride may carry appreciable quantities of boron (table 1). It was suggested [4] that the boron in this reagent probably comes from the glaze of the vessels in which it is dehydrated. Accordingly, boron from this source would appear for the most part on the surface of the granules, and its removal should be possible by extraction or volatilization. Nearly all the boron can be removed (table 1) in a single treatment by drenching 500 g of the salt in a liter copper beaker with 300 ml of 95-percent methyl alcohol containing 15 ml of concentrated hydrochloric acid and heating the well-stirred mixture on a hot plate and finally in an oven at 200° C. until the alcohol is expelled and the material again becomes anhydrous.

Absolute methyl alcohol for use in the isolation of boron from the sample was prepared by redistillation of absolute alcohol from a 3-liter Pyrex flask to which had been added a few sticks of potassium hydroxide. The presence of volatile organic acids in the alcohol would give high results for boron by the usual titration with two indicators (*13, p. 614*), and accordingly the 4.5 parts per million of B_2O_3 found in methyl alcohol as received (table 1) might be regarded merely as the B_2O_3 equivalent of the alkali consumed by such organic acids. However, the titration procedure used by the authors eliminates from the boron titration all acids that do not form complexes with mannitol, and, therefore, it would appear that boron was actually present in the alcohol. In any event the redistilled alcohol was very satisfactory.

As a matter of convenience a solution of mannitol, rather than the solid, was used in the titrations. The solution was prepared by dissolving 100 g of mannitol in recently boiled distilled water and making the volume up to 1 liter.

The sodium carbonate was used as received. Any boron in the hydrochloric acid would appear in the results for sodium hydroxide; however, the indications were that the acid contained considerably less boron than did the sodium hydroxide.

TITRATION OF BORIC ACID

In figure 1 are reproduced Foote's titration curves (*9*) for a buffered water (I) and the same containing added boric acid with (III) and without (II) mannitol. Accordingly, if the solution containing boric acid without mannitol is titrated (adjusted) until its pH value reaches some point *B* (curve II) in the pH range over which curves II and III are nearly parallel—7.6 in the present instance—a part of the boric acid will have been titrated, the alkali equivalent being *A′B′*. Then, on the addition of mannitol the pH value of the solution drops to *B″* on curve III, and the titration can be completed by adding alkali (*B′ C′* ml) until the pH value of the solution is again 7.6 at point *C*. The total alkali equivalent of boric acid is *A′ C′*, but since point *A″* on curve II cannot, in general, be determined because of the limitations of indicators, as well as the presence of other acids that titrate in this pH range, the total equivalent has no meaning in actual practice.

[4] WICHERS, E. Private communication. National Bureau of Standards.

The problem, therefore, resides in the choice of the pH value to which the solution shall be adjusted before mannitol is added. For this purpose various indicators have been used; mention may be made of methyl red and paranitrophenol. With the former, the pH value lies at some point *E* on curve II; with the latter, at some point *D*. Since a different indicator, usually phenolphthalein, is used for the final titration, alkali is consumed in bringing a boron-free solution from the end point of one indicator to that of the other, which increases the blank correction to the titration. For example, Allen and Zies (*2*, *p.*

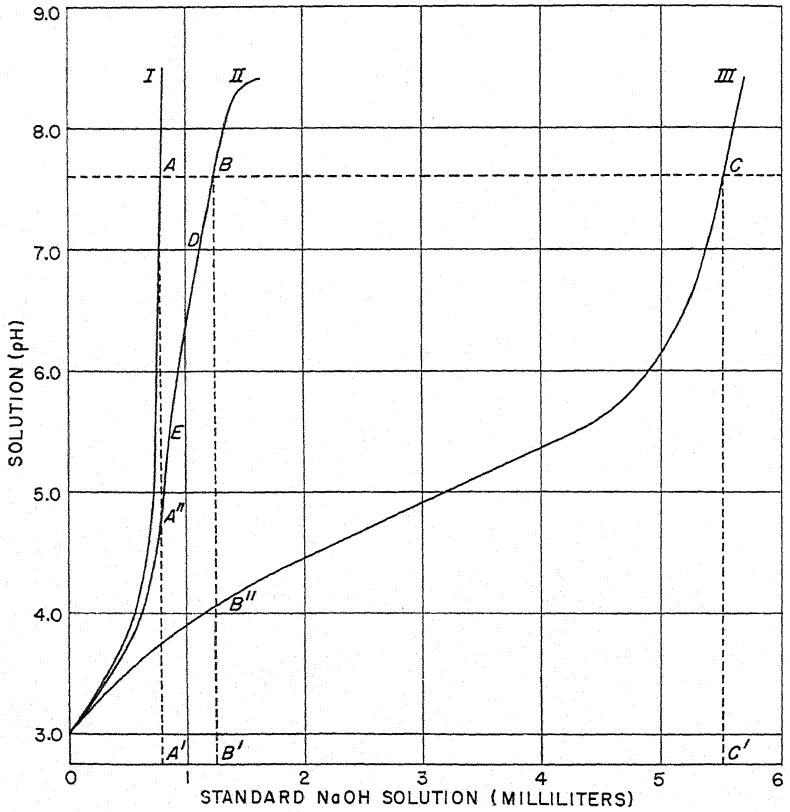


FIGURE 1.—Titration curves for boric acid (*9*). Curve I is for 50 ml of water, curve II for 50 ml of water plus 7.4 mg of B_2O_3, curve III for 50 ml of water plus 7.4 mg of B_2O_3 and 6 g of mannitol.

765) found the B_2O_3 equivalent of the alkali used in passing from the paranitrophenol end point to that of phenolphthalein to be 0.3 mg, which corresponds to a little less than 0.1 ml of 0.1 *M* sodium hydroxide, and the writers found the amount of alkali consumed between the methyl red and phenolphthalein end points to be 0.23 ml of 0.03 *M* sodium hydroxide. Furthermore, in the presence of weak acids or substances that titrate as weak acids, which are not readily separated from boric acid, as, for example, soluble fluosilicate, adjustment of the solution to a pH value in the approximate range *ED* with the necessary accuracy is extremely difficult, if not impossible. Moreover, the

titration of these same substances is continued after the addition of mannitol, thereby vitiating the result for boric acid.

The foregoing difficulties are obviated in Foote's method for the direct titration of boric acid in water (*9*) by adjusting the pH value of the solution before the addition of mannitol to the same value as that of the final end point. Since the two end points involve no pH change, other substances in solution do not interfere with the titration, except insofar as they render the end point difficult to establish. The small amount of boric acid neutralized during the initial adjustment ($A'B'$, fig. 1) is accounted for in the standardization factor of the alkali, and therefore the alkali equivalent used in practice is $B'C'$. Carbon dioxide, of course, must be excluded from the titration.

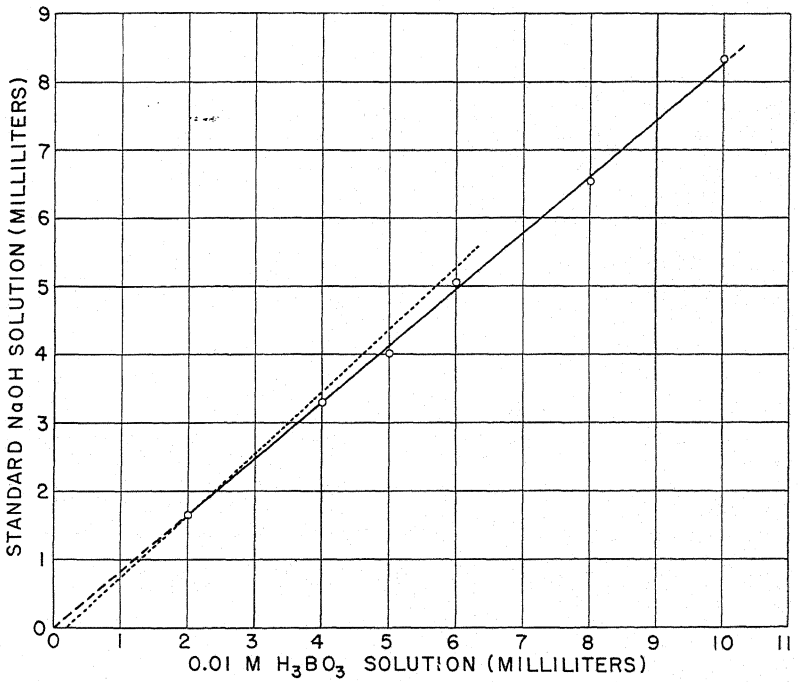


FIGURE 2.—Curve obtained in standardization of approximately 0.01 *M* sodium hydroxide against a 0.01 *M* boric acid solution with use of phenolphthalein as the indicator of both end points (10° C.). Volume of solution at initial end point was 100 ml, that at final end point was 125 ml plus the volume of standard sodium hydroxide added.

Foote adjusted his solutions to a pH of 7.6 colorimetrically with phenol red, and Wilcox (*25*) used an electrometric method. The authors used phenolphthalein, because of the comparative ease with which the end point can be recognized without accessory equipment. According to measurements with the glass electrode,[5] the faint pink of the phenolphthalein indicator in the concentration used by the authors appeared at the unusually low pH of about 7.6. In figure 2

[5] According to measurements made by L. M. White of the Fertilizer Research Division, the pH at which the faint pink color appeared in 100 ml of a 0.4 *M* solution of sodium chloride containing 20 drops of a 2-percent phenolphthalein solution was 7.6; with 10 drops of indicator the pH was 7.7, whereas with only 1 drop it was 8.4

are shown the results obtained in a typical standardization of sodium hydroxide solution against a boric acid solution prepared from fused pure boric acid (*13, p. 613*). The results fall on a straight line with a maximum deviation of about ±0.1 ml of 0.01 *M* alkali. Since the curve passes through the origin, the blank correction is zero, as it should be with boron-free alkali, and the B_2O_3 equivalent of the alkali is obtainable directly from the slope of the curve. With an alkali containing an appreciable quantity of boron the curve would be rotated in the direction of the dotted line (fig. 2).

Besides carbon dioxide, which must always be excluded in the titration of boric acid, other known constituents of the analytical solutions obtained from natural phosphates that are likely to interfere in the subsequent titration of the boron are arsenic and fluorine. Allen and Zies (*2, p. 767*) found that the presence of 1 mg of As_2O_3 did not affect the titration of boric acid, but the presence of 25 mg rendered the phenolphthalein end point indistinct because of fading. The quantities of arsenic reported in natural phosphate (*15, p. 30*) are usually less than 0.005 percent of As_2O_3, which corresponds with 0.5 mg of As_2O_3 per 10 g of sample. Thus, if all the arsenic in the sample should follow the boron, the quantity present in the solution for titration would still be well within the tolerance indicated by Allen and Zies' results.

Quadrivalent germanium (*22*) and hexavalent tellurium (*20*) form complexes with mannitol that titrate as monobasic acids. Tellurium, like selenium, appears to be reduced to the metal in the presence of methyl alcohol, and thus it does not accompany boron in the alcohol distillate.[6] Although germanium behaves toward mannitol almost exactly as does boron, only a small fraction (probably 10 percent) of the amount of this element in the sample accompanies the boron in the final solution.[6] Thus, if the presence of germanium be neglected, the error thereby introduced in the result for boron (B_2O_3) would, because of the difference between the atomic weights of these elements, be equivalent to one-third of the germanium (GeO_2) accompanying the boron, or probably 3 to 4 percent of the germanium in the sample. Germanium has not been detected in phosphate rock, but, as far as the authors know, only two samples have been examined for this element (*14*).

The effect of fluoride and fluosilicate on the titration of boric acid apparently has not been studied to any extent heretofore. Allen and Zies (*2, p. 769*) noted some difficulty with the phenolphthalein end point in the titration of a solution obtained by distilling a synthetic mixture of boric acid and 0.2 g of NaF, whereas in a similar experiment with less fluorine (0.1 g of KF) Chapin (*23, p. 1691*) encountered no difficulty. The authors studied the effect of the presence of fluorine in quantities up to 100 mg by the addition of aliquots of a solution of sodium fluoride or hydrofluosilicic acid[7] to aliquots of standard boric acid solution and titration of the resultant solution for boron with phenolphthalein as previously indicated.

No interference was observed with sodium fluoride in the absence of notable quantities of free silica. On the other hand, with hydrofluosilicic acid pronounced fading of the indicator occurred at the

[6] HAGUE, JOHN L. Private communication. National Bureau of Standards.

[7] Prepared in the laboratory by adding an excess of pure quartz flour to c. p. hydrofluoric acid contained in a platinum dish, allowing the mixture to stand until the solution was clear, and decanting into a wax bottle.

final end point, and the result for boric acid tended to be decidedly low. This behavior is attributed to the re-formation of some fluosilicic acid as a consequence of the acidity developed in the boron-containing solution when mannitol is added to it. During the subsequent titration, the decomposition of the re-formed fluosilicic acid, which takes place slowly at the low temperature of the titration, would cause fading of the indicator and, unless the titration is continued until all the fluosilicic acid is destroyed, also a low titer for boric acid. The error arising from this source can be almost completely eliminated by continuing to add alkali until the pink of the indicator persists in the solution for at least 1 minute.

SEPARATION OF BORON FROM THE SAMPLE

The authors' recovery of added boron from synthetic mixtures and phosphate rock low in aluminum by distillation with phosphoric acid and hydrochloric acid is inclined to be 1 to 5 percent low as shown in table 2. Aluminum compounds show a marked tendency to prevent the distillation of boron, an observation also noted by others.[8] Early in the experiment, the use of phosphoric acid was discontinued because the results for acid-soluble boron obtained on phosphate rock with it were less than half the values obtained with hydrochloric acid. Later, however, it was discovered that if the rock-acid mixture is allowed to stand several hours before distillation, phosphoric acid alone gives as good results as hydrochloric acid and calcium chloride, provided only a very small quantity of water is present. For the distillation of samples that have been fused with sodium carbonate a larger quantity of phosphoric acid is needed to take care of the several milliliters of water added with the decomposed melt. The addition of phosphoric oxide (*6*) would seem preferable to the addition of larger quantities of acid.

TABLE 2.—*Recovery of boron by acid distillation*

Tabulation No.	Distillation mixture	B_2O_3			Fluorine present during titration
		Present	Found [1]	Recovered	
		Milligrams	*Milligrams*	*Percent*	*Milligrams*
1	10 ml 0.01 *M* H_3BO_3+10 ml concentrated H_3PO_4.	3.48	[2] 3.31–3.44	95.0–98.8	0
2	10 ml 0.03 *M* H_3BO_3+20 ml concentrated H_3PO_4	10.44	10.13–10.31	97.0–98.8	0
3	1 ml 0.1 *M* H_3BO_3+20 ml concentrated HCl+20 g $CaCl_2$.	3.48	3.34	96.0	0
4	10 g phosphate rock No. 912+20 ml concentrated H_3PO_4.	----	[3] .34	----	147
5	10 g phosphate rock No. 912+20 ml concentrated HCl+20 g $CaCl_2$.	----	.33	----	67
6	Residue in distillation flask from 5+1 ml 0.1 *M* H_3BO_3.	3.48	3.52	100	3
7	10 g phosphate rock No. 912+1 ml 0.1 *M* H_3BO_3+20 ml concentrated HCl+20 g $CaCl_2$.	[4] 3.81	3.62	95.0	97
8	Same as 7+2 g Al_2O_3	[4] 3.81	3.13	82.2	2
9	10 g No.[5] 904+1 ml 0.1 *M* H_3BO_3+20 ml concentrated HCl+20 g $CaCl_2$.	3.48	2.90	83.3	0

[1] 400 ml of distillate were collected.
[2] The first 200 ml of distillate contained 3.20 to 3.34 mg of B_2O_3, and the second 200 ml 0.06 to 0.12 mg.
[3] Rock and acid were mixed and let stand overnight before distillation.
[4] Added B_2O_3 plus B_2O_3 found in 10 g of No. 912 (tabulation No. 5).
[5] Natural aluminum phosphate.

[8] ZIES, E. G. Private communication. Geophysical Laboratory, Carnegie Institution of Washington.

Relatively large quantities of fluorine and silica usually follow the boron. The observed quantities of fluorine were indeed variable and ranged from 2 to 147 mg (table 2), depending, among other things, on the quantity of acid used, the temperature in the distilling flask, and the composition of the sample. When 20 ml of hydrochloric acid were used in the distillation the quantity of fluorine following the boron usually ranged from 40 to 90 mg.

Since fluorine in the presence of silica has an adverse effect on the titration of boric acid, some simple means of separation is highly desirable. The addition of Al_2O_3 to the distilling flask is not permissible because it holds back the boron as well as the fluorine. Double distillation with hydrochloric acid reduced the amount of fluorine in the distillate about 50 percent, and double distillation in which only 3 to 5 ml of excess hydrochloric acid and 0.05 g of $ZrOCl_2$ were added to the flask before redistilling the second time lowered it still further to 10 mg or less without affecting the results for boron. The somewhat increased precision thereby obtained was not, in the author's opinion, sufficient to justify the additional time required for analysis.

Total boron, as distinguished from acid-soluble boron obtained by direct distillation of the phosphate sample, was determined in a few typical phosphate rocks by fusing the sample with twice its weight of sodium carbonate prior to distillation (*13, p. 614*). The results by the two methods of decomposition are shown in table 3. Obviously all the boron is not, in general, completely expelled from the sample by direct treatment with acid under the conditions that prevail in the distillation. The results by the two methods on Florida land pebble and two of the three Tennessee brown rocks are in substantial agreement, whereas in other instances (except No. 904) the results for total boron are considerably higher than those for acid-soluble boron. In the case of highly aluminiferous materials, such as soft phosphate (No. 580), the difference between the results is probably attributable to the retention of boron under certain conditions by aluminum compounds, as was noted in a preceding section. Available information relating to the boron-bearing constituents of phosphates does not justify any explanation for the disparity in the results for the other phosphates (table 3). It may be merely coincidental that the latter contain either relatively large quantities of organic matter (No. 1253), or sulphide minerals (Nos. 56 and 930), or both (No. 948).

TABLE 3.—*Boron in typical phosphate rocks as determined in fused and unfused samples*

Sample No.	Source or type of phosphate	Al_2O_3	B_2O_3 determined in sample—	
			Fused with Na_2CO_3	Unfused
		Percent	*P. p. m.*	*P. p. m.*
120 [1]	Florida land pebble	0.82	24	20
912	do	1.05	28	33
908	Tennessee brown rock	1.16	40	48
1253	Idaho rock	1.16	86	45
948	Wyoming rock	1.19	107	60
930	Tennessee blue rock	1.22	81	65
56a [1]	Tennessee brown rock	1.99	54	48
56 [1]	do	3.06	77	51
580	Florida soft	23.05	60	18
904 [2]	Grand Connetable Island	36.92	<10	<10

[1] Standard sample number of the National Bureau of Standards.
[2] Natural aluminum phosphate.

PROCEDURE USED

Add 20 ml of concentrated hydrochloric acid to 10 g of the sample in a liter flask of Kavalier glass. Swirl the flask until the contents are well mixed and then allow it to stand from ½ to 1 hour with occasional swirling. At the end of this period add 20 g of purified calcium chloride to the flask and mix by swirling; then add 50 ml of redistilled absolute methyl alcohol, and after mixing the contents by rapid swirling connect the reaction flask to the distilling apparatus (*13, p. 613*). Heat the reaction flask with a free flame until the contents begin to boil, then swirl the flask a few times without disconnecting it from the condenser, and replace the flame with a water bath maintained at 80° to 90° C. for the duration of the distillation. Keep the volume of alcohol in the reaction flask as constant as possible by distilling directly into it alcohol from a second distilling flask, which should be heated to boiling temperature before inserting the reaction flask to the system. Should the reaction flask run dry, the results will be low and erratic. Regulate the rate of distillation so that 400 ml of distillate will be obtained in 45 to 60 minutes.

Receive the distillate in a 500-ml Kavalier flask containing 30 ml of distilled water and 6 ml of 6 *M* sodium hydroxide solution, and stop the distillation when the contents of the receiver reach a volume of about 450 ml. When the distillation is finished add to the distillate 0.5 ml (10 drops) of a 2-percent solution of phenolphthalein and additional 6 *M* sodium hydroxide solution as needed to develop a permanent deep pink color.

If the sodium hydroxide carries an appreciable quantity of boron it will be necessary to measure the volume of alkali used, in order to make the proper blank correction to the result for boron. Accordingly, it is convenient to add the 6 *M* alkali in 6-ml portions. Ordinarily the alkali initially added to the receiving vessel is sufficient, though in runs on aliquots of a solution of boric acid alone and not infrequently with rock samples an additional 6 ml is required, and occasionally, when an unusually large quantity of fluorine distills over, as much as 18 ml of alkali will be needed.

After the addition of an excess of alkali to the distillate, distill off the alcohol by immersing the flask in a water bath with due care to avoid superheating at the beginning of distillation. Boiling tubes are indispensable here. When alcohol no longer distills over, transfer the pink residual aqueous solution directly to a 300-ml Kavalier flask for titration. It is not necessary to evaporate the solution to dryness and ignite the residue, as is done in the analysis of plant materials (*24*). Adjust the volume of the solution to 100 ml with water and boil the alkaline solution from 4 to 5 minutes on a hot plate to expel any residual alcohol before it is made acid incident to the removal of carbonate.

Render the hot alcohol-free solution acid with hydrochloric acid by adding concentrated acid until nearly all the excess alkali is neutralized, then 0.1 *M* acid to neutrality, and finally about 1 ml of 0.1 *M* acid in excess. Boil the acidified solution about 3 minutes with several vigorous swirlings during this period; make the solution faintly alkaline with 1 *M* alkali, then acid with 0.1 *M* hydrochloric acid, using 6 drops in excess, and reboil as before. Again restore the pink

color of the indicator with alkali, discharge it with 0.1 *M* acid, using this time only 2 drops in excess, boil again, and then cool the nearly neutral carbonate-free solution to 10° C. by placing the stoppered flask in a bath of ice water.

It is considered good practice to guard against a possible loss of boron during the boiling of the acidified solution by connecting the flask to an air condenser. The stepwise approach to the neutral point with intervening boiling not only increases the precision of the method but also lowers the blank titration on the reagents.

Adjust the cold (10° C.) milky solution accurately to the initial end point by adding 0.03 *M* sodium hydroxide solution to a permanent faint pink color, keeping the flask stoppered as far as possible to prevent the ingress of carbon dioxide, then add 25 ml of a 10 percent solution of mannitol. Boil the solution from 3 to 4 minutes with occasional swirling, again cool it to 10° C., and titrate it at this temperature with 0.03 *M* sodium hydroxide solution until the faint pink of the indicator persists for at least 1 minute. The result for boric oxide is obtained by multiplying the volume of sodium hydroxide solution (corrected for the blank titration on the reagents) consumed in the titration after the addition of mannitol by the factor for the alkali found by standardization against pure boric acid, the same titration procedure being used.

When only 6 ml of 6 *M* alkali was added to the alcohol distillate, the blank titration on the reagents amounted to 0.1 ml of 0.03 *M* sodium hydroxide, or 13 p. p. m. of B_2O_3 on the basis of 10 g of sample, which agrees well with the value (11 p. p. m.) expected from the analyses of the purified reagents (table 1). Furthermore, in the presence of the usual quantities of fluorine and silica the precision of the titration is also about 0.1 ml of 0.03 *M* sodium hydroxide. Accordingly, when the corrected titer was only 0.1 ml, the result is arbitrarily reported as $\leqq$10 p. p. m., whereas with a smaller titer the result is reported as $<$10 p. p. m.

Duplicate determinations of boron in the fluorine-bearing phosphates usually agreed within 15 p. p. m., the average difference being 8 p. p. m. Agreement was closer in the case of fluorine-free materials.

BORON IN NATURAL PHOSPHATES

Results for acid-soluble boron in domestic and foreign phosphates are given in tables 4 and 5, respectively. For the few results for total boron, as determined by fusing the sample with sodium carbonate, reference must be made to table 3. The data in tables 4 and 5 are summarized according to type of phosphate in table 6. South Carolina rock ranks first in quantity of boron and the African rocks second, each of these types of phosphate averaging more than 90 p. p. m. of B_2O_3. At the other extreme is bone ash, Florida soft and waste-pond phosphates, and Russian apatite, with less than 20 p. p. m. With the exception of the phosphatic limestone the averages for all other types of phosphate examined range from 42 to 61 p. p. m. of B_2O_3, inclusive.

TABLE 4.—*Acid-soluble boron in domestic phosphates*

FLORIDA PHOSPHATES

Sample No.	Type of phosphate	Location of deposit	P_2O_5	B_2O_3
			Percent	*P. p. m.*
618	Land pebble	Pierce	30.53	98
619	do	Nichols	30.98	67
910	do	Mulberry	31.09	75
947	do	Brewster	31.28	72
790	do	Not known	31.40	70
912	do	Mulberry	35.37	33
120 [1]	do	do	35.40	20
1447 [2]	do	Bartow	35.11	54
1310 [3]	do	Not known	34.43	63
771	Hard rock	do	31.25	60
932	do	Dunnellon	35.99	40
580	Soft	Not known	29.49	18
728	do	Juliette	31.80	<10
581	Waste pond	Not known	18.18	<10
915	do	Dunnellon	23.63	<10

SOUTH CAROLINA PHOSPHATES

495	Not known	Not known	16.07	63
1139	Land rock	Bulow mines, Johns Island	26.92	126
1138	do	Lamb's mine, near Cherleston	27.85	142
1137	do	do	27.58	144

TENNESSEE PHOSPHATES

56 [1]	Brown rock	Not known	31.28	51
56a [1]	do	Mount Pleasant	32.94	48
762	do	do	33.72	53
906	do	Wales	34.39	36
908	do	Mount Pleasant	34.44	48
1401 [3]	do	Wales	36.73	70
772	Blue rock	Glover	30.45	66
930	do	Gordonsburg	30.97	65
1049	Kidney	Boma	31.22	56
1048	White rock	Tomscreek	30.20	55
1031	do	Godwin	35.80	57
917	Phosphatic limestone	Gordonsburg	11.68	30

WESTERN PHOSPHATES

550	Light colored	Idaho, Paris	32.21	67
1011	do	Montana, Garrison	27.63	32
1009	do	do	31.39	22
1252	do	do	36.38	48
1010	do	do	37.47	40
948	Dark colored	Wyoming, Cokeville	30.19	60
973	do	Idaho, Conda	32.53	70
1253	do	do	32.13	45

OTHER PHOSPHATES

1295	Apatite	Virginia, Amherst County	39.58	47
971	Bone ash		40.36	17

[1] Standard sample number of the National Bureau of Standards.
[2] Concentrated by oiling and tabling.
[3] Concentrated by froth flotation.

TABLE 5.—*Acid-soluble boron in foreign phosphates*

AFRICAN PHOSPHATES

Sample No.	Location of deposit	P_2O_5	B_2O_3
		Percent	*P. p. m.*
1559	Algeria, Djebel Kouif	30.00	116
1549	Egypt, Safaga	32.79	110
593	Morocco	34.30	66
1551	Tunis, Gafsa	29.05	84

INSULAR PHOSPHATES

452	Christmas Island	39.46	46
985	Curaçao Island	38.59	70
904 [1]	Grand Connetable Island	54.51	<10
1230	Makatea Island	37.94	72
450	Nauru Island	38.92	55
451	Ocean Island	40.32	37

APATITES

905	Canada, Quebec Province	40.30	≧10
1305	Union of Soviet Socialist Republics, Kola Peninsula	31.63	12
1304	do	37.39	20

[1] Natural aluminum phosphate.

TABLE 6.—*Acid-soluble boron in different types of natural phosphates*

DOMESTIC PHOSPHATES

Type or source of phosphate	Samples analyzed	B_2O_3	
		Range	Average
	Number	*P. p. m.*	*P. p. m.*
Florida land pebble	9	20–98	61
Florida hard rock	2	40–60	50
Florida soft and waste pond	4	<10–18	<12
South Carolina	4	63–144	119
Tennessee brown rock	6	36–70	51
Tennessee blue and white rocks and kidney phosphate	5	55–66	60
Tennessee phosphatic limestone	1	----------	30
Western part of the United States:			
Light-colored rock	5	22–67	42
Dark-colored rock	3	45–70	58
Apatite from Virginia	1	----------	47
Bone ash	1	----------	17

FOREIGN PHOSPHATES

Africa	4	66–116	94
Islands	[1] 5	37–72	56
Apatite from the Union of Soviet Socialist Republics	2	12–20	16

[1] Excluding the sample (No. 904) of natural aluminum phosphate.

The authors' results for boron in phosphate rock are considerably lower than most of the figures reported in the literature. Young (*26*, *p. 32*), using the official method (*3*, *p. 32*) for acid-soluble boron, found 450 p. p. m. of B_2O_3 in a sample of Florida hard rock, only traces in two other Florida phosphates of unknown type, and 450 and 580 p. p. m. in two Tennessee brown rocks. The spectrochemical results of Gaddum and Rogers (*10*) are much higher. Thus, the latter authors report the equivalent of 0.16 to 1.6 percent of B_2O_3 in Florida pebble and soft phosphates. On the other hand, recent spectrochemical anlyses, made with extreme care by B. F. Scribner of the National Bureau of Standards (*14*), showed only 0.005 percent of B_2O_3 and <0.001 percent of B_2O_3, respectively, in a Tennessee brown rock (National Bureau of Standards standard sample No. 56a) and a

Florida land pebble (National Bureau of Standards standard sample No. 120). The results agree well with the authors' figures for these samples as shown in table 4.

At present, little can be said concerning the boron-bearing minerals occurring in natural phosphates. Schaller (*21, p. 168*) has prepared a list of all the known boron minerals including 56 names comprised in 2 groups, viz, 36 borates and 20 borosilicates. Many of these minerals are soluble in water and a large majority of them are either soluble in, or are decomposed by, hydrochloric acid. Several of the borosilicates, as, for example, tourmaline, are not decomposed by hydrochloric acid, though in the analysis of phosphate rock their decomposition would be promoted by the fluorine present in the sample. So far as the authors are aware, only one boron mineral has been identified in phosphate rock. A very small quantity of tourmaline was found (*15, p. 76*) in one sample of Florida land pebble (No. 912).

BORON IN SUPERPHOSPHATES AND DEFLUORINATED PHOSPHATE ROCK

In view of the presence of considerable quantities of fluorine in most natural phosphates and the volatility of boron fluoride, it would be difficult to predict the fate of the boron in phosphate rock during the manufacture and storage of superphosphate. In general, one might expect the boron to distribute itself between the superphosphate and the gases expelled when the rock and acid are mixed and during subsequent storage of the superphosphate. On the other hand, if boron-free acid were used and no boron were volatilized during manufacture, the boron content of the resulting superphosphate would, in the case of ordinary superphosphate, be roughly 50 percent of that of the ingredient rock. Thus, on the basis of the data in table 6, ordinary superphosphates prepared from Florida land pebble and Tennessee brown rock, respectively, would probably contain 10 to 50 and 18 to 35 p. p. m. of B_2O_3. These figures agree reasonably well with the amounts found by analysis (table 7).

TABLE 7.—*Acid-soluble boron in superphosphates and defluorinated phosphate rocks*

SUPERPHOSPHATES

Sample No.	Phosphatic fertilizer		P_2O_5	B_2O_3
	Type	Made from—		
			Percent	*P. p. m.*
1403 [1]	Ordinary superphosphate	Florida land pebble	20.56	<10
1402 [2]	do	do	20.60	<10
1580	do	do	20.85	51
1581	do	Tennessee brown rock	20.24	36
1486	Special superphosphate [3]	Florida land pebble	32.67	58
1361	Double superphosphate	do	49.27	94
1481	do	Tennessee brown rock	43.43	77
1362	do	do	48.37	158
1372	do	Idaho rock	47.33	96

DEFLUORINATED PHOSPHATE ROCKS

1476 [4]	Fused phosphate rock	Tennessee brown rock	28.95	21
1351	Calcined phosphate rock	do	36.58	20
1478	do	do	37.25	30

[1] The sulphuric acid used in the preparation of this superphosphate was processed sludge acid from the raffination of asphalt-base petroleum.

[2] The sulphuric acid used in the preparation of this superphosphate was untreated sludge acid from the raffination of asphalt-base petroleum.

[3] Prepared by granulating a mixture of ordinary and double superphosphate.

[4] Defluorinated in the fused condition (7).

The agreement of the results in the foregoing comparison relating to ordinary superphosphate leaves the inference that in the manufacture of phosphoric acid by the wet process a large part of the boron in the phosphate rock may go into the product acid. Accordingly, double superphosphate made with such phosphoric acid would be expected to contain as much, or more, boron than the ingredient rock. The results for boron in double superphosphates (table 7), in comparison with the boron content of the same types of phosphate rock (table 6), clearly support this expectation.

Results for boron in domestic superphosphates reported by previous workers (*10, 26*) are considerably higher than the figures obtained by the authors, the differences being about the same as were found in the case of phosphate rock.

Without asserting that previously reported results are subject to such error, it should be pointed out in this connection that superphosphate, because of the presence of active fluorine compounds, readily attacks glass and cannot be stored in glass bottles without becoming contaminated with boron from the container. For the sake of comparison, results on portions of double superphosphates No. 1361 and 1362 that had stood in glass bottles for 3 years may be cited. Samples taken from the center of these bottles, that is, out of direct contact with the glass, showed 120 and 186 p. p. m. of B_2O_3, respectively, as compared with 94 and 158 p. p. m. (table 7) in material that was stored in wooden containers.

The quantity of acid-soluble boron found in defluorinated Tennessee brown-rock phosphate (*7, 16*) amounted to 20 to 30 p. p. m. of B_2O_3 (table 7), and since this material is almost completely decomposed by hydrochloric acid, these figures may be regarded as a close approach to the total boron. On the basis of the range of the results for brown rock (table 6) it appears that around 50 percent of the boron in phosphate rock is volatilized in the defluorination process. Defluorinated phosphate rock probably carries less boron than basic slag. An imported slag (not shown in the tables), the only slag examined by the authors, contained 116 p. p. m. of B_2O_3.

SUMMARY

The Chapin method, modified to the extent that only one indicator is required in the titration, was applied to the determination of the relatively small quantities of boron occurring in natural phosphates and superphosphates.

Results are given for boron in 54 samples of natural phosphates from various deposits of the world, 9 samples of superphosphates, and 3 samples of defluorinated phosphate rock. The results for acid-soluble boron (B_2O_3) in natural phosphates range from <10 to 144 p. p. m., in superphosphates from <10 to 158 p. p. m. and in defluorinated phosphate rock from 20 to 30 p. p. m.

LITERATURE CITED

(1) ANONYMOUS.
1935–36. BORON AND PLANT LIFE. Fert., Feed. Stuffs, Farm Supplies Jour. 20: 720–722, 724, 748–752, illus., 1935; 21: 4–6, 8, 60–62, 64, illus., 1936.

(2) ALLEN, E. T., and ZIES, E. G.
1918. A CONTRIBUTION TO THE METHODS OF GLASS ANALYSIS, WITH SPECIAL REFERENCE TO BORIC ACID AND THE TWO OXIDES OF ARSENIC. Jour. Amer. Ceram. Soc. 1: [739]–786, illus.
(3) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.
1935. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. . . . Ed. 4, 710 pp., illus. Washington, D. C.
(4) BERTRAM, P.
1935. DIE BEDEUTUNG EINIGER SPURELEMENTE FUER DIE PFLANZEN. 4th Cong. Internatl. Tech. et Chim. des Indus. Agr. Bruxelles, v. 2, pp. 396–414, illus.
(5) BRECKPOT, RAYMOND.
1935. DOSAGE SPECTROGRAPHIQUE DE CERTAINS CONSTITUANTS SECONDAIRES DU NITRATE DE SOUDE DU CHILI. 4th Cong. Internatl. Tech. et Chim. des Indus. Agr. Bruxelles, v. 2, pp. 382–388, illus.
(6) CALFEE, R. K., and MCHARGUE, J. S.
1937. OPTICAL SPECTROGRAPHIC DETERMINATION OF BORON: POLARIZING ATTACHMENTS. Indus. and Engin. Chem., Analyt. Ed. 9: 288–290, illus.
(7) CURTIS, HARRY A., COPSON, RAYMOND L., BROWN, EARL H., and POLE, GORDON R.
1937. FERTILIZER FROM ROCK PHOSPHATE: CONVERSION BY FUSION AND TREATMENT WITH WATER VAPOR. Indus. and Engin. Chem. 29: 766–770, illus.
(8) EATON, FRANK M.
1935. BORON IN SOILS AND IRRIGATION WATERS AND ITS EFFECT ON PLANTS WITH PARTICULAR REFERENCE TO THE SAN JOAQUIN VALLEY OF CALIFORNIA. U. S. Dept. Agr. Tech. Bull. 448, 132 pp., illus.
(9) FOOTE, FRED J.
1932. DETERMINATION OF BORON IN WATERS: METHOD FOR DIRECT TITRATION OF BORIC ACID. Indus. and Engin. Chem., Analyt. Ed. 4: 39–42, illus.
(10) GADDUM, L. W., and ROGERS, L. H.
1936. A STUDY OF SOME TRACE ELEMENTS IN FERTILIZER MATERIALS. Fla. Agr. Expt. Sta. Bull. 290, 15 pp.
(11) HANCE, F. E.
1933. REPORT ON CHEMISTRY. Hawaii. Sugar Planters Assoc. Proc. 53 (Rept. Com. in Charge Expt. Sta.): 46–63.
(12) HILL, H.
1936. MINOR ELEMENTS AFFECTING HORTICULTURAL CROPS. Sci. Agr. 17: 148–153, illus.
(13) HILLEBRAND, W. F., and LUNDELL, G. E. F.
1929. APPLIED INORGANIC ANALYSIS; WITH SPECIAL REFERENCE TO THE ANALYSIS OF METALS, MINERALS, AND ROCKS. 929 pp., illus. New York and London.
(14) HOFFMAN, JAMES I., and LUNDELL, G. E. F.
1938. THE ANALYSIS OF PHOSPHATE ROCK. Natl. Bur. Standards Jour. Research 20 (5): 607–626, illus.
(15) JACOB, K. D., HILL, W. L., MARSHALL, H. L., and REYNOLDS, D. S.
1933. THE COMPOSITION AND DISTRIBUTION OF PHOSPHATE ROCK WITH SPECIAL REFERENCE TO THE UNITED STATES. U. S. Dept. Agr. Tech. Bull. 364, 90 pp.
(16) ——— REYNOLDS, D. S., and MARSHALL, H. L.
1936. PHOSPHATE FERTILIZERS BY CALCINATION PROCESS—VOLATILIZATION OF FLUORINE FROM PHOSPHATE ROCK AT HIGH TEMPERATURES. Amer. Inst. Mining and Metall. Engin. Tech. Pub. 695, 14 pp., illus.
(17) KERN, EDWARD F., and JONES, THOMAS R.
1930. TITRATION OF FLUOSILICIC AND FLUOBORIC ACIDS. Amer. Electrochem. Soc. Trans. 57: 273–278, illus.
(18) MARSHALL, H. L.
1934. THE OCCURRENCE OF FERROUS IRON IN PHOSPHATE ROCK. Jour. Agr. Research 49: 71–76.

(19) RADER, LEWIS F., Jr., and HILL, W. L.
1935. OCCURRENCE OF SELENIUM IN NATURAL PHOSPHATES, SUPERPHOSPHATES AND PHOSPHORIC ACID. Jour. Agr. Research 51: 1071–1083.

(20) ROSENHEIM, ARTHUR, and WEINHEBER, M.
1911. ZUR GRAVIMETRISCHEN BESTIMMUNG DES TELLURS UND ALKALIMETRISCHEN BESTIMMUNG DER TELLURSÄURE. Ztschr. Anorgan. Chem. 69: 266–269.

(21) SCHALLER, WALDEMAR T.
1930. BORATE MINERALS FROM THE KRAMER DISTRICT, MOHAVE DESERT, CALIFORNIA. U. S. Geol. Survey Prof. Paper (1929) 158: 137–170, illus.

(22) TCHAKIRIAN, ARAKEL.
1928. DOSAGE TITRIMÉTRIQUE DE L'ACIDE GERMANIQUE, ÉTUDES DE QUELQUES FORMES HYDRATÉES DE CET ACIDE ET DE SES SELS. Compt. Rend. Acad. Sci. [Paris] 187: 229–231.

(23) WHERRY, EDGAR T., and CHAPIN, W. H.
1908. DETERMINATION OF BORIC ACID IN INSOLUBLE SILICATES. Jour. Amer. Chem. Soc. 30: 1687–1701, illus.

(24) WILCOX, L. V.
1930. DETERMINATION OF BORON IN NATURAL WATERS AND PLANT MATERIALS. Indus. and Engin. Chem., Analyt. Ed. 2: 358–361, illus.

(25) ———
1932. ELECTROMETRIC TITRATION OF BORIC ACID. Indus. and Engin. Chem., Analyt. Ed. 4: 38–39.

(26) YOUNG, R. S.
1935. CERTAIN RARER ELEMENTS IN SOILS AND FERTILIZERS, AND THEIR RÔLE IN PLANT GROWTH. N. Y. (Cornell) Agr. Expt. Sta. Mem. 174, 70 pp.

THE ANATOMICAL DEVELOPMENT OF LEPIDIUM DRABA[1]

By AUSTIN O. SIMONDS[2]

Assistant in botany, Colorado Agricultural Experiment Station

INTRODUCTION

The difficulties of weed control suggest the need for a fuller knowledge of the life histories of these plants. One of the most persistent perennial weeds in the West is hoary cress (*Lepidium draba* L.). In an effort to determine the cause of its resistance to eradication, a study was made of its anatomical development from the seed to the mature plant. The results are presented in this report.

MATERIALS AND METHODS

The studies of the anatomy of the seedling were made with plants grown from seeds secured from the Colorado seed laboratory and from seeds obtained from mature plants in the field. The mature plants used for the anatomical studies and those used as a source of seed were collected along a road and in a nearby field north of Loveland Lake, Loveland, Colo.

The studies on seedling development were made on plants collected weekly up to 6 weeks of age, then at 2 months, 3 months, 4½, and 5½ months. The plants 3 and 5½ months old were grown both in a garden plot and in the greenhouse. Plants of corresponding ages from the greenhouse and outdoor plots showed very few outstanding differences.

The paraffin method was used in most of the work, although free-hand sections were studied to check certain structures. Most seedlings were killed and fixed in chromo-acetic-formalin (42 cc of 1-percent chromic acid, 3½ cc of glacial acetic acid, 15½ cc of formaldehyde, and 39 cc of water). Alcohol-formalin-acetic fluid (48 cc of alcohol, 40 cc of water, 7 cc of formaldehyde, and 5 cc of glacial acetic acid) was used for the mature plant tissues. Because of the abundance of stone cells in the root and crown, and the large amount of lignified tissues in the older organs, it was necessary to treat these materials in hydrofluoric acid for 1 week before they were processed for sectioning in paraffin. Safranin-fast green was the stain combination used most frequently; safranin-Mayer's haem-alum was also employed.

EXTERNAL MORPHOLOGY OF THE SEEDLING

The cotyledons appear above ground 5 or 6 days after planting. The cotyledons expand in about 3 weeks to maximum size, approximately five times their dimensions in the seed. During this interval the first leaves emerge and begin to enlarge (fig. 1, 1–5). The leaves continue to enlarge for 5 or 6 weeks and form a loose rosette.

[1] Received for publication March 14, 1938; issued February 1938. Contribution from the Department of Botany, Colorado Agricultural Experiment Station.

[2] The author wishes to express his appreciation to Dr. L. W. Durrell, head of the Botany Department of Colorado State College, for consultation and for advice pertaining to these investigations, and to Dr. J. E. Sass and Dr. J. N. Martin of Iowa State College for help and suggestions in the preparation of the manuscript.

The radicle develops into a prominent taproot, from which lateral roots arise in 2 to 3 weeks. An enlarged region at the base of the hypocotyl, referred to as the "collet," marks the junction of the root and hypocotyl.

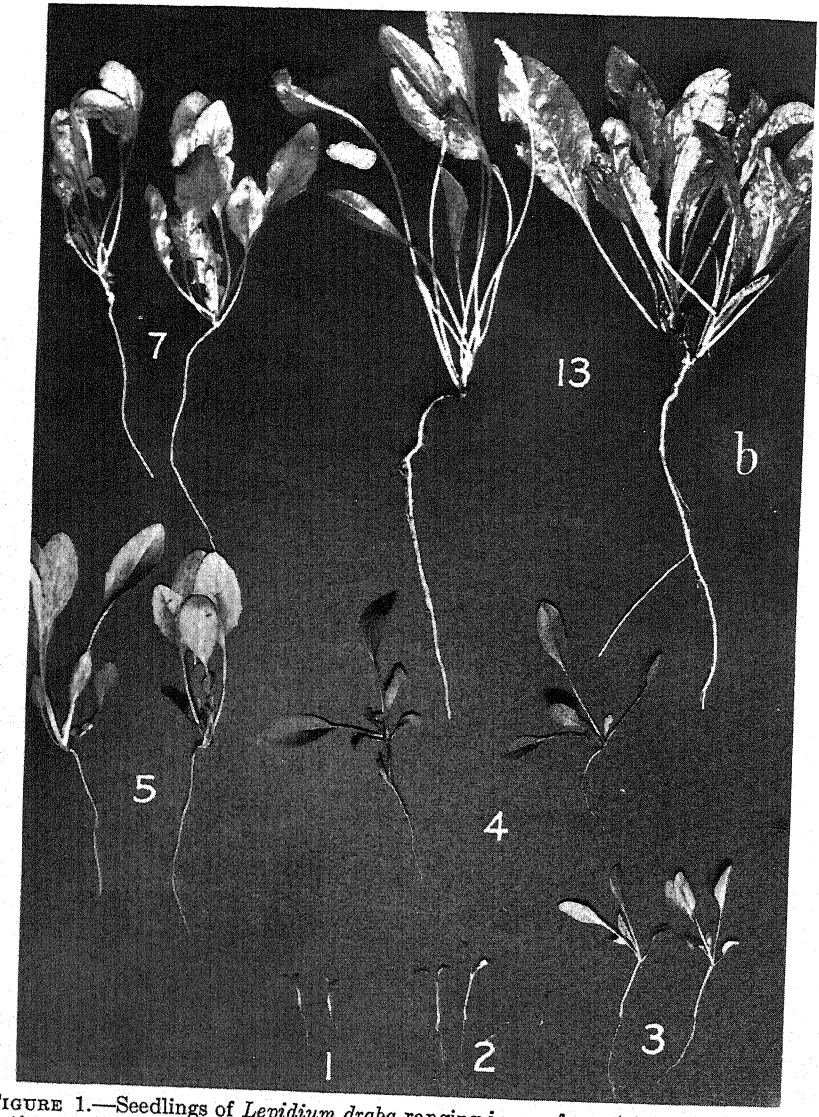


FIGURE 1.—Seedlings of *Lepidium draba* ranging in age from 1 to 13 weeks from time of planting seed, as indicated by numerals; *b* shows stem shoots arising from the hypocotyl.

Lateral stem shoots begin to appear in about 13 or 14 weeks, arising very often from the upper regions of the hypocotyl (fig. 2) and from the cotyledonary axils of the older plants. After this stage the plant begins to assume more mature vegetative characteristics. A descrip-

tion of the mature plant will not be given here, since it has been adequately described by others.[3]

ANATOMY OF THE EMBRYO

Preliminary to the embryonic studies, the seeds were soaked for a time in order to remove the seed coats, and the extracted embryos were embedded and sectioned in paraffin. Transverse and longitudinal sections (pl. 1, *A* and *B*) show that the cotyledons are reflexed nearly parallel to the radicle. The tip of the radicle has a thin root cap formed from a calyptrogen. The apical meristem of the epicotyl, between the curved cotyledons, consists of a small, conical mass of

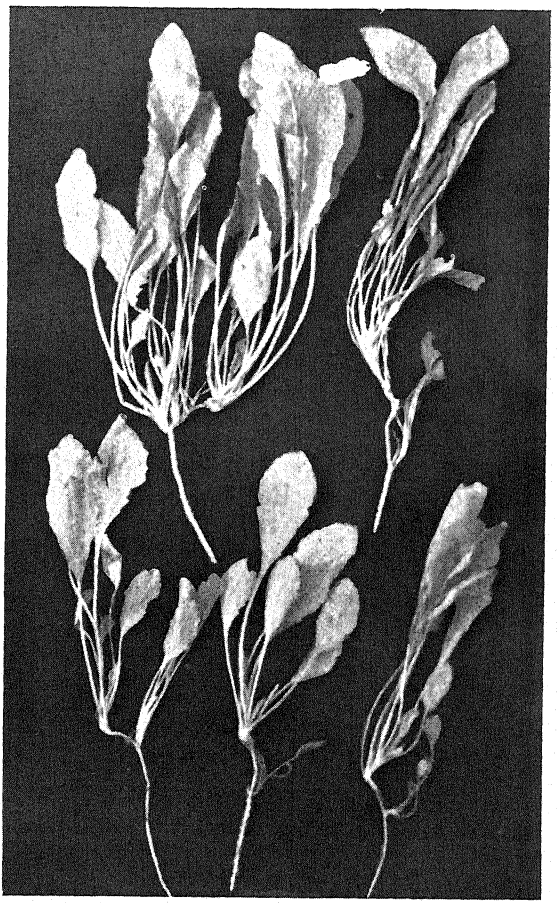


FIGURE 2.—Seedlings of *Lepidium draba* showing secondary shoots arising from the hypocotyl region.

[3] HITCHCOCK, C. LEO. THE GENUS LEPIDIUM IN THE UNITED STATES. Madroño 3: 265-320, illus. 1936.
BELLUE, MARGARET K. NEW WEEDS CONFUSED WITH HOARY CRESS. Calif. Dept. Agr. Monthly Bull. 22: 288-293, illus. 1933.
PEITERSEN, A. K., and BURDICK, R. T. PERENNIAL PEPPERGRASS—A NOXIOUS WEED IN COLORADO. Colo. Expt. Sta. Bull. 264, 10 pp., illus. 1920.

cells. Some cells of the embryo appear to have two nuclei, and most of the cells contain an abundance of starch.

The central provascular cylinder of the radicle and hypocotyl branches just below the cotyledonary node and provides a branch for each of the cotyledons (pl. 1, *B*). The internal (ventral) surface of the cotyledons contains cells which form a palisadelike layer. The cells of the dorsal region are also very compact but more irregularly arranged.

THE ROOT

THE PRIMARY ROOT

The primary vascular system in the young seedling is a diarch protostele of the exarch type (pl. 1, *C*). In plants 1 week old, only the protoxylem areas are lignified. At this age the metaxylem cells are of mature size but are not lignified. Two small protophloem areas are present but are distinguished with difficulty from the pericycle. In plants at least 2 weeks old the phloem cells are usually smaller than those of the pericycle, and some sieve tubes and companion cells are recognizable.

The pericycle consists of one layer of parenchymatous cells. A supplementary layer of interstitial parenchyma fills in the area between the protoxylem and protophloem. The cells of the endodermis in the root of the 10-day-old plants are conspicuously larger than the pericyclic cells and may be recognized by the narrow elliptical casparian strips (pl. 2, *B*). Casparian strips were observed only in the lower part of the hypocotyl. The endodermal cells are thin-walled, very similar to the cortical cells although not quite so large in diameter.

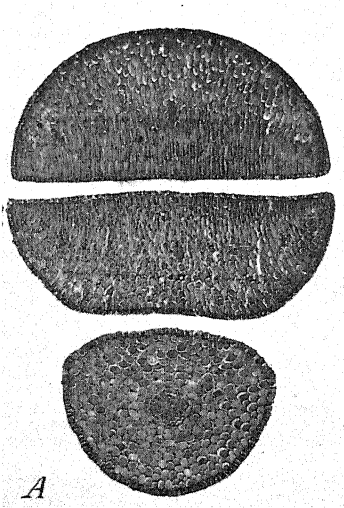
The wide cortex of the primary root consists of parenchymatous cells with large intercellular spaces. The very loose arrangement of the cells is conducive to the subsequent rapid break-down of the cortex. The epidermal cells are thin-walled and parenchymatous, varying in cross-sectional diameter both radially and tangentially In the region of absorption, root hairs are formed but are of short duration because of the early sloughing of the cortex.

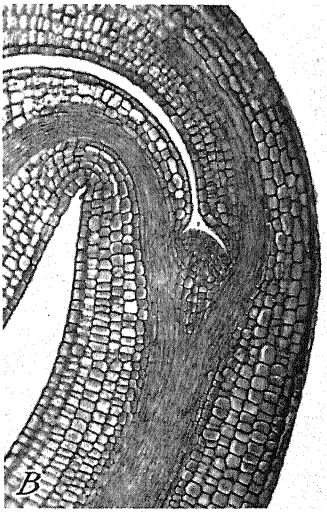
DEVELOPMENT OF THE ROOT

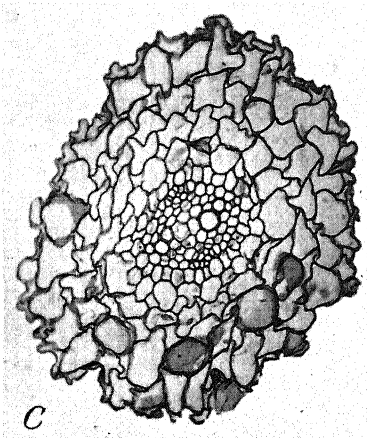
Structure of the Xylem

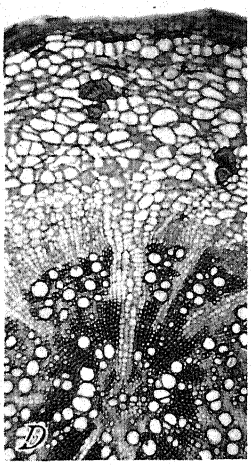
The formation of secondary xylem is initiated by a cambium between the small masses of primary phloem and xylem. The first secondary xylem cells are formed at right angles to and nearest the center of the diarch primary xylem (pl. 1, *C, D*). As development proceeds, fusiform cambial cells originate in the parenchymatous tissue tangential to those already present, gradually filling out the central, somewhat circular, area of xylem. After the first-formed masses of secondary xylem are as long as or slightly longer than the protoxylem areas on a radial plane, the cambium connects around the two points of primary xylem.

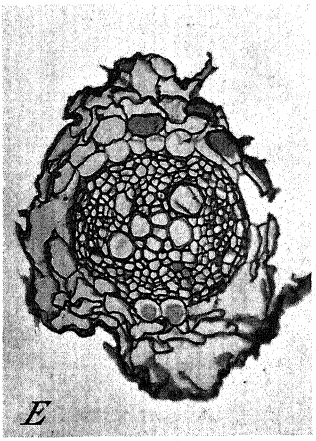
The lignified tracheae are interspersed among the parenchymatous cells and fiber tracheids. Extending radially from the protoxylem areas, wide parenchymatous xylem rays are laid down by the cambium. Narrow xylem rays also occur in the lignified areas of the xylem. The origin of these later-formed rays apparently is at the beginning of a new season of growth (pl. 1, *D*).

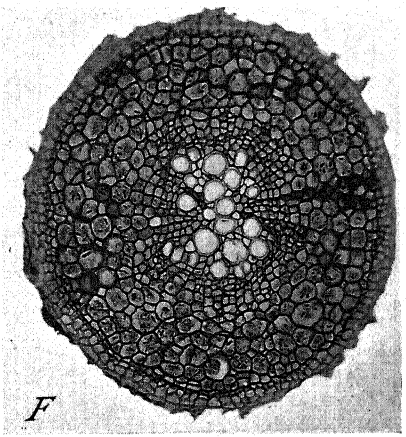




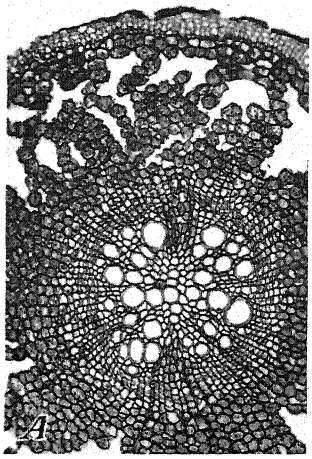


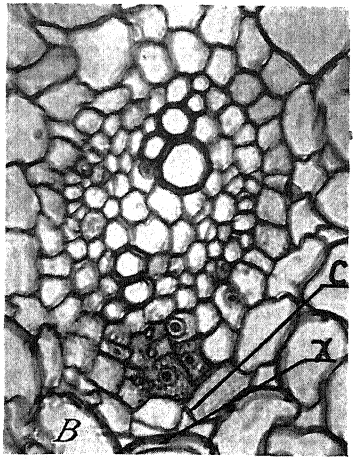


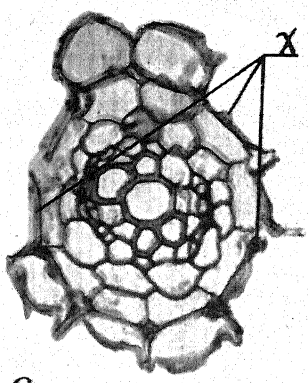




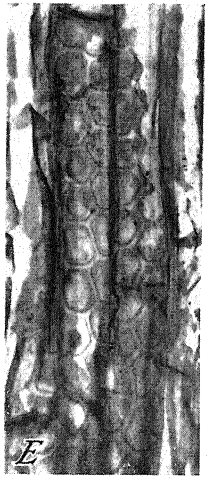
A, Cross section of seed embryo. × 40. *B*, Longitudinal section of seed through apical meristem. × 77. *C*, Section of week-old root. × 147. *D*. Section of 2-vear-old root from field. × 31. *E*. Section of 4-week-old

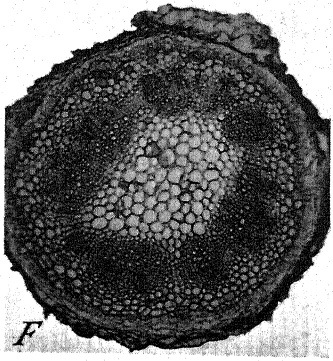


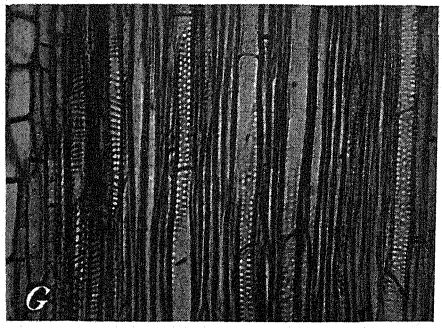












A, Section about 2 inches below ground line of a 90-day-old root from garden plot. × 65. *B*, Section of 10-day-old seedling root. × 394. *C*, Section of small lateral root from 18½-week-old plant showing the inner cortical reticulum in cross section. × 485. *D*, Lignified corticular reticulum in innermost cortical layer from 18½-week-old primary root. × 418. *E*, Lignified corticular reticulum in innermost cortical layer from 12-week-old primary root. × 423. *F*, Section through one of the lower internodes of 18½-week-old stem. × 38. *G*, Longitudinal section of portion of vascular area in a mature stem. × 134.

Longitudinal sections of young and old roots show that the first protoxylem elements consist mostly of spiral tracheae, although some annular cells are formed. The secondary wall thickenings of these cells are very delicate. In the tracheae that are produced later, the secondary wall consists of pronounced scalariform thickenings. It was observed that a few elements formed at the end of the growing season were more like reticulate cells. The fiber tracheids have sharper pointed ends than typical tracheids, smaller lumina than the smallest tracheae, and greatly reduced bordered pits. The xylem parenchyma cells of the old roots are from two to five times as long as they are wide, and the majority of the cells have transverse or nearly transverse end walls.

Annual growth rings are formed in the portion of the root a few inches below the surface of the ground. The "springwood" contains relatively large tracheae and considerable parenchyma, whereas the "summerwood" contains smaller tracheae and a band of xylem fibers. Thus the difference in spring and summer growth makes the rings apparent.

Structure of the Phloem

The formation of secondary phloem is slow as compared with the rate of xylem formation. The phloem cells differ from the pericycle in that their walls appear a little thicker in cross section and stain slightly darker. The sieve tubes were found to have nearly transverse end walls, with the sieve plates located only at the ends. The angular companion cells are of the same length as the sieve tubes, very small in diameter, and have protoplasm slightly more dense than that of the sieve tubes.

The cambium produces a large amount of phloem parenchyma in which sieve tubes and companion cells are found in small isolated strands. However, stages were found where the phloem conducting tissue was difficult to recognize because of a wide radial band of undifferentiated cambial derivatives.

The older conducting phloem becomes crushed and is absorbed in the surrounding tissues as the plant axis continues to increase in diameter. This condition was observed in some sections from plants 12 weeks or more of age (pl. 1, *D*).

The Cortex

Very early in the life of the plant the cortex becomes wrinkled, breaks down, and sloughs away (pl. 1 *C*, *E*, and *F*). This breaking-down process is sometimes associated with the differentiation of a characteristic structure in the first layer of cortical cells next to the endodermis. A reticulate tertiary thickening is deposited on the inside of the inner tangential wall in these cells (pl. 2, *B*, *C*, *D*, and *E*). Not all young roots sectioned showed this structure. The youngest was 10 days old and the oldest was 18½ weeks old.

The thickenings stain red like lignified material when safranin and fast green are used as a staining combination. The meshes of the reticulum spread out flat in each cell and sometimes the open ends in two adjacent cells are opposite each other. This netlike structure was found only in the very young primary roots and in young secondary roots. It appeared to completely surround the stele of the

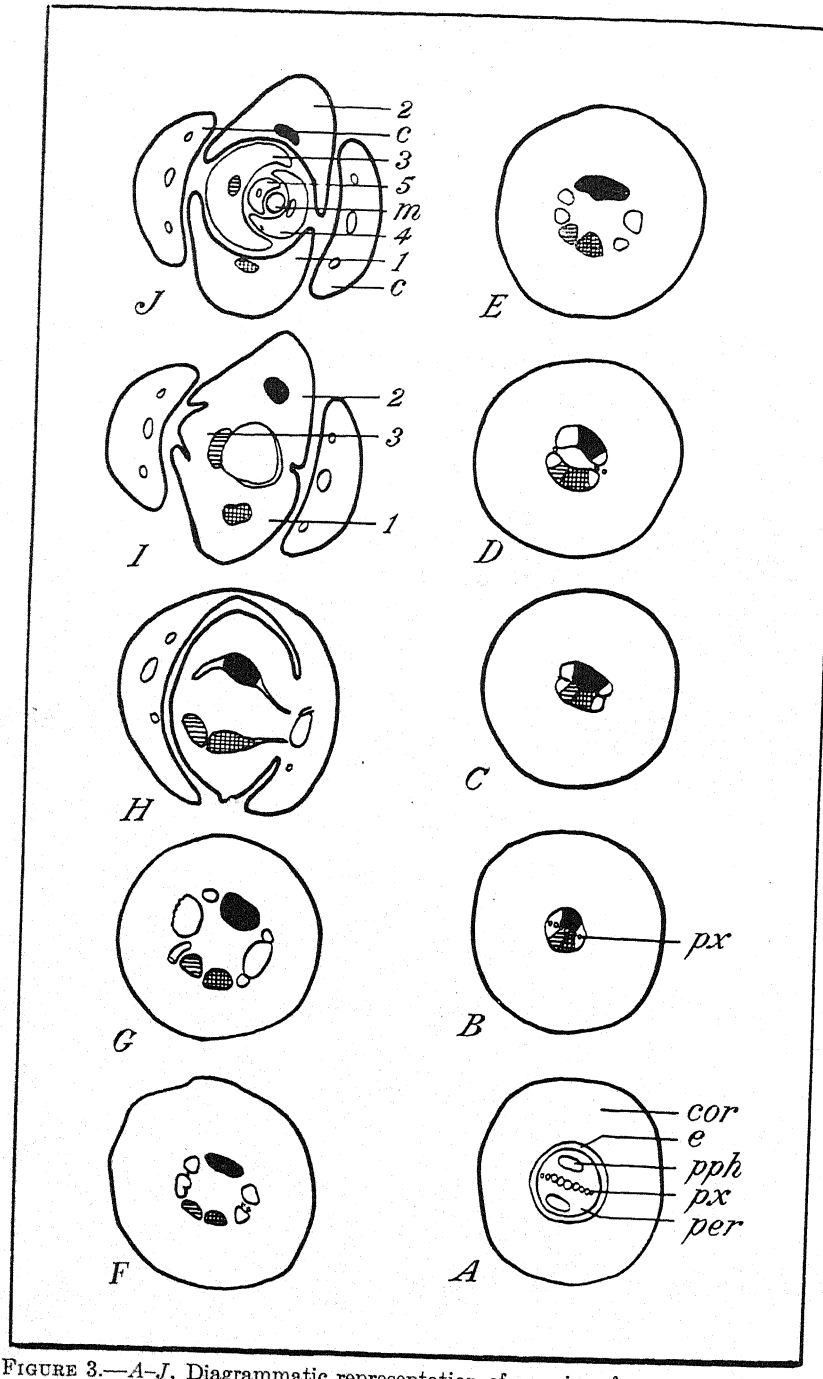


FIGURE 3.—*A–J*, Diagrammatic representation of a series of cross sections from a 3-weeks-old plant of *Lepidium draba* showing how each vascular trace of the young seedling arises from the stele below. In *I* the numbers 1, 2, and 3, and in *J* numbers 1 to 5 indicate the bases of the first three and the first five leaves, respectively. *cor*, cortex; *e*, endodermis; *pph*, primary phloem; *px*, primary xylem; *per*, pericycle; *c*, cotyledons; *m*, meristems.

roots of some young plants, while still younger roots had only a small patch on one side (pl. 2, *B*).

A periderm arises in the pericycle and is followed by the breaking down of the cortex. The phellogen produces a layer of cork which may attain a thickness of 12 or more layers of cells in old roots. However, the unbroken inner layer was only four or five cell layers in thickness. No evidence of the production of a phelloderm was found, all the tissue external to the cambium of the old root apparently being made up of secondary phloem cells.

Small clusters of these phloem cells in the "cortical region" of the root differentiate into stone cells as secondary phloem sclerenchyma. Plants taken from the garden plot October 10 showed stone-cell differentiation taking place, and most of the sections of old roots had stone cells in this tissue (pl. 1, *D*). The vascular tissues of the phloem are separated by phloem rays.

The 90-day-old root (pl. 2, *A*) had a considerable number of intercellular spaces in the "cortical region." These cavities extend vertically in the root for some distance. The exact length could not be determined from longitudinal sections because the cavities are not perfectly parallel with the axis.

Sections taken from roots 12 inches below the ground line did not contain as many of the intercellular spaces as sections from roots at 4- and 6-inch depths; while at 22 inches down, very small and few air passages occurred.

ROOT-STEM TRANSITION

The root-stem transition is most easily understood by studying seedling plants. The diarch protostele vascular arrangement of the root continues up into the hypocotyl to within a few millimeters of the cotyledonary node. The first indications of the transition change from root to stem are the production of slightly larger masses of secondary tissue situated at right angles to the line of the primary xylem, and the collapse of some of the primary xylem cells (fig. 3, *C*). A close examination of the center of a section, taken at a slightly higher level, shows the presence of two central parenchyma cells which is the first indication of the pith and of the siphonostele of the stem (fig. 3, *D*). Slightly larger masses of secondary tissue are also present and the primary xylem is reduced on each side to two tracheae.

A section taken from the hypocotyl about one-half millimeter higher has the secondary tissue divided into seven small areas separated by parenchyma cells. The secondary tissue contiguous to each side of the protoxylem points becomes separate from the semicircular masses, forming four bundles, represented as clear areas (Fig. 3, *E*). The secondary tissue on one side remains in one bundle and that on the opposite side separates into two smaller bundles. Figure 3, *F*, is similar to *E*, differing only in the fact that *F* shows the first indication of the separation of a small mass of vascular tissue to form a lateral cotyledonary trace. This can be seen as a partly divided bundle (clear) at the left side of figure 3, *F*.

No evidence of the primary vascular system can be found at the plane where the section for figure 3, *G*, was taken. The two small bundles at the sides of the primary xylem points fuse and send out small bundles which will become lateral bundles of the cotyledons.

The changes that take place at successive levels are evident in figure 3, *H*, *I*, and *J*. In *I* the central vascular ring shown in *H* has divided to become the vascular traces for the first three leaves. The section cut through the apical meristem (fig. 3, *J*) shows the cotyledonary bases at the outside extremities with the base of five leaves in between them and the meristematic tip in the center.

THE STEM

PRIMARY STEM STRUCTURES

Since the hypocotyl up to a few millimeters below the cotyledons is a diarch protostele like the root already described, a detailed description of the hypocotyl will not be necessary. The principal anatomical difference between the root and hypocotyl is that the cortical region of the hypocotyl is larger in diameter than that of the root and its epidermis contains stomata. The root-stem transition, which takes place a short distance below the cotyledons, has been described.

A prominent shoot is not evident until the plants are at least 12 weeks old. The protoxylem consists largely of spiral elements, although a few annular and scalariform protoxylem cells are formed. The metaxylem contains a few reticulate and pitted elements. The first-formed protophloem appears to be phloem parenchyma, although very early the sieve tubes and companion cells are in evidence in small restricted patches.

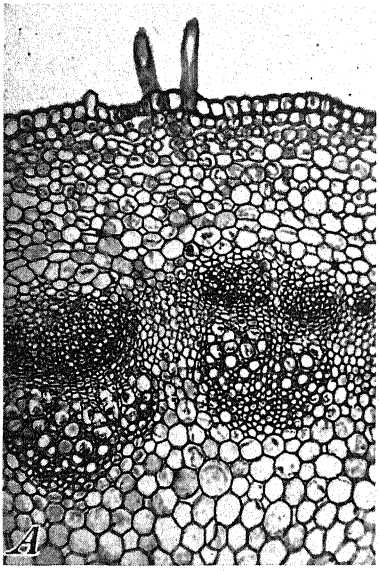
A cross section of an internode of an 18½-week-old stem discloses a large pith with large intercellular spaces and an irregular medullary sheath adjacent to the primary xylem (pl. 2, *F*). The vascular cylinder consists of vascular bundles, some of which are distinct while others are joined by vascular tissues formed by the interfascicular cambium.

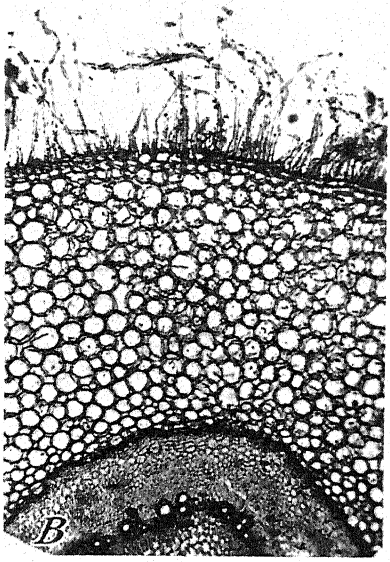
The xylem is made up of small clusters of lignified tracheae interspersed among parenchymatous cells and partially differentiated fibers. The cambium cells are small, thin-walled, and somewhat rectangular in cross section. The phloem at this age consists of sieve tubes, clearly defined companion cells, and undifferentiated fiber cells.

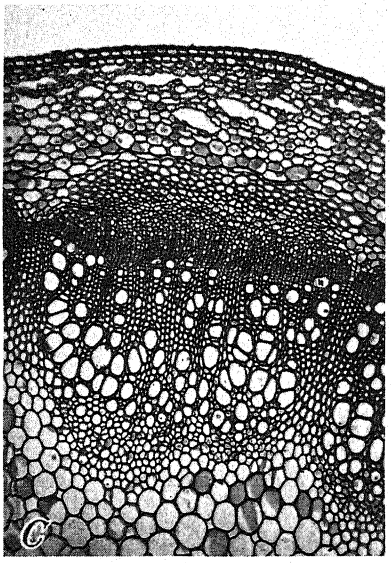
In many of the plants grown in greenhouse flats and in some of the stem shoots of field plants the cortex of the stem near the ground had collapsed and disintegrated. This condition was found in plants growing in a crowded environment. Under such conditions, a periderm was found just outside the phloem, probably arising in the innermost part of the cortex. Thus the "cortical region" of such an organ (pl. 2, *F*) is largely made up of old phloem, limited on the outside by the thin periderm layer. The cortex higher up on the stem is not sloughed off but remains as a loosely arranged tissue with large intercellular spaces (pl. 3, *A*).

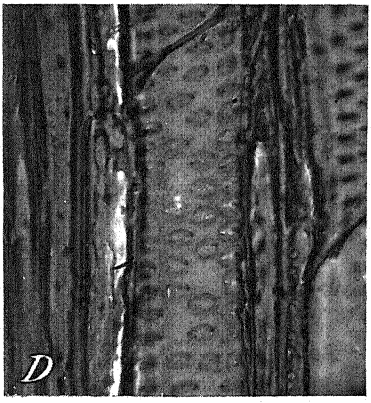
CHANGES IN THE MATURE STEM

The more mature stem structures can be seen in plate 3, *C*. Besides the noticeable increase in vascular tissue certain other changes are apparent. In the cortex of the stem the intercellular passages are larger and appear to be more numerous than in the younger stem. These passages are arranged in such a way as to give a netlike appearance to regions of the cortex. The epidermal cells of the stem appear

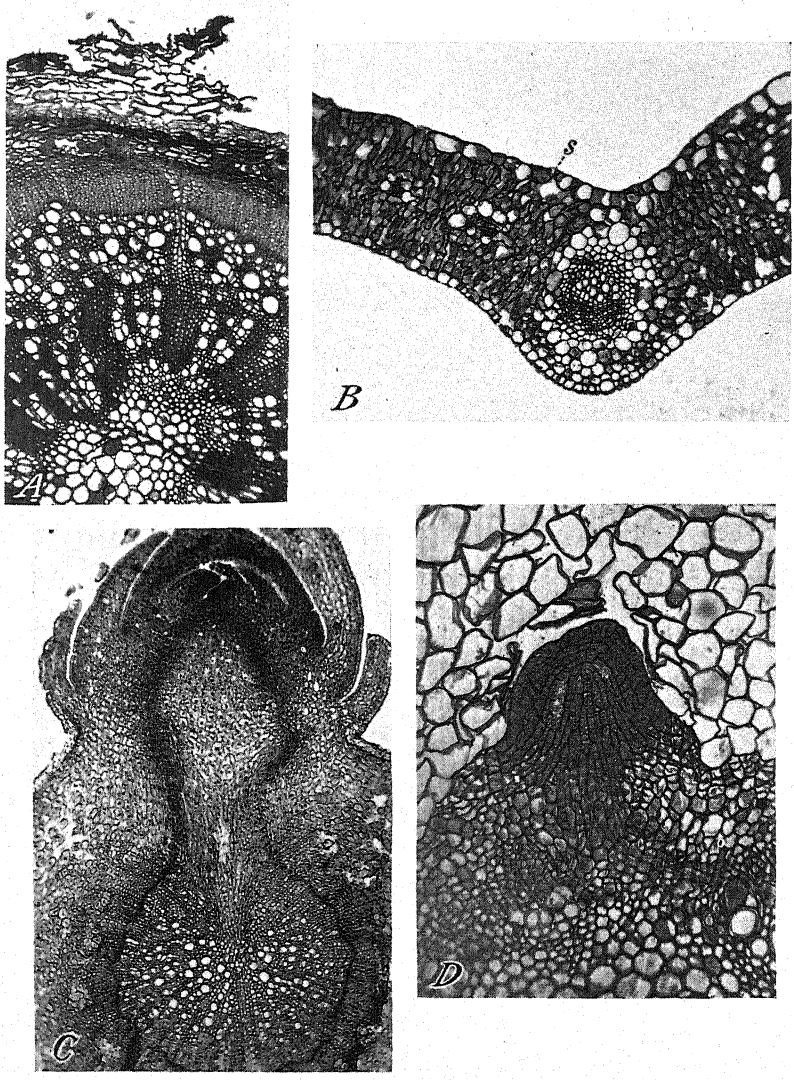








A, Section of young stem. × 96. *B*, Section from below ground of young stem shoot showing stem hairs modified as root hairs. × 43. *C*, Section of mature stem. × 75. *D*, Longitudinal section of pitted trachea showing perforated torus. × 708.



A, Section of crown showing the lignified rays. × 58. *B*, Section of mature leaf. × 67. *C*, Section through a root showing stem shoot arising from the root. × 46. *D*, Section through stem shoot showing the origin of an adventitious root. × 101.

rectangular from external view and are nearly isodiametric in cross section. These thick-walled cells are cutinized on both the inner and outer tangential walls. A slightly thinner-walled hypodermis was evident on the mature stem (pl. 3, *C*). The guard cells of the stomata are small and slightly sunken. Their cross-sectional area was about one-fourth that of the normal epidermal cells in close proximity to them.

Epidermal hairs, uniform in shape, are commonly present over most of the aerial organs of the plant. Their structure will be described later as part of the leaf. The hairs on the subterranean portion of young stem shoots just emerging from the ground in moist soil are more numerous and longer but otherwise similar to root hairs (pl. 3, *B*).

These modified epidermal hairs were not equally abundant around the entire circumference of the stem. They were longer and thicker on the lower side of the bend where the shoots were attached to the old crown. Since these hairs were found early in the spring and were not present on older shoots later in the growing season, they are considered to be temporary structures. The indications are that under the environmental conditions encountered early in the spring (April 15), these modified stem hairs function as root hairs for a time, but as the top layer of the soil dries out and a periderm develops in the pericycle of these underground organs, cutting off the outer tissues, the cells dry up and disappear.

THE CROWN

During a severe winter the crown and even some of the top of the root may be killed. When this occurs, new crown shoots are formed as adventitious buds near the top of the living section of root. In the plots where the tops of the roots were dug for anatomical studies, from one to four new shoots were sent up through 12 inches of soil. The lower 9 or 10 inches of these shoots developed crown characteristics and the remaining portion had stem structure.

Since the early development of the crown was found to be similar to that of the aerial stem, the development of the two organs will not be discussed separately. Both young stem and crown have a ring of vascular bundles forming a dissected siphonostele, with rays of parenchyma connecting the pith with the cortex. In the crown cambial activity is more rapid than in the stem, resulting in the early formation of a continuous cylinder of thick-walled lignified xylem (pl. 4, *A*).

As in the root, the primary cortex of the crown is sloughed off early in the life of the organ. A phellogen arising in the tissues outside the primary phloem forms a protective layer of phellem. Since the endodermis could not be identified in the crown, the exact tissue giving rise to the phellogen was not determined. Judging from the amount of parenchyma cells inside the periderm, the phellogen in the crown probably arises in the primary cortex.

The crown of old plants develops clusters of stone cells in the parenchyma outside the active phloem early in April (pl. 4, *A*), whereas the seedling plants in deep greenhouse flats did not form the stone cells until August, when the plants were 6 months old. Intercellular spaces were formed in the outer portion of old phloem of the crown the first year in plants grown both in the greenhouse and in the garden plot.

Comparisons of old crowns with the lower part of mature stems did not reveal any striking structural differences. In the upper levels of the mature stem isolated masses of fibers occur just outside the active phloem (pl. 3, *C*). The pith of the stem is much larger in cross section

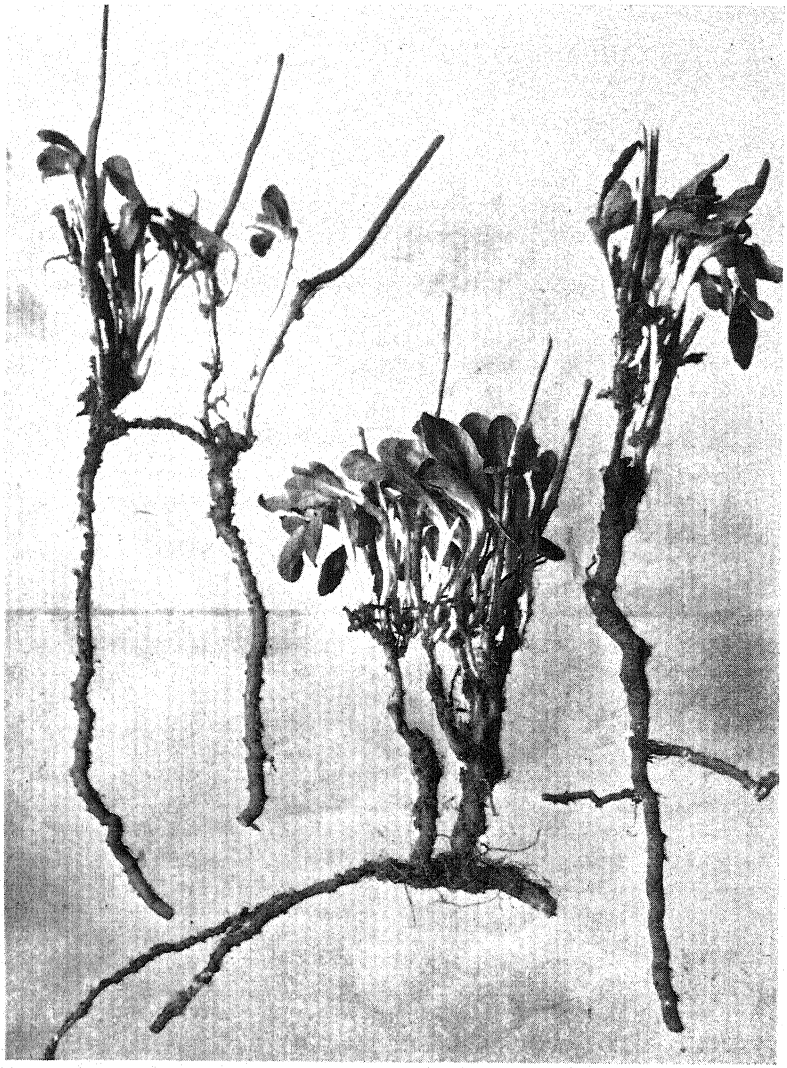


FIGURE 4.—*Lepidium draba,* showing new stem shoots arising from the crown shoots and also a portion of the root system.

than the pith of the crown. The stem contains tracheids, whereas the crown had thick-walled, long fiber tracheids with sharp tapered ends. The pitted tracheae of the crown and stem have large pit openings, which when greatly magnified show the torus to be perforated (pls. 3, *D* and 2, *G*).

ADVENTITIOUS SHOOT AND ROOT FORMATION

The root system of the plant has both perpendicular and horizontal roots (figs. 4 and 5). No structural differences were found between the horizontal and the perpendicular roots and both produce adventitious buds which develop into shoots. Under field conditions the horizontal roots send up the most shoots and thus are the principal means of vegetative spread of the plant. If the top of a vertical root is killed by winter freezing or is cut off in digging, new aerial shoots arise opposite a large xylem ray in the phloem. Plate 4, *C*, shows a shoot arising from an old root.

In the seedling stage, supplementary stem shoots commonly arise from the middle or lower portion of the seedling hypocotyl (fig. 2 and fig. 1, 13, *b*). Since that part of the hypocotyl was found to be a protostele similar to the root, the formation of these buds is similar to buds formed on the vertical roots.

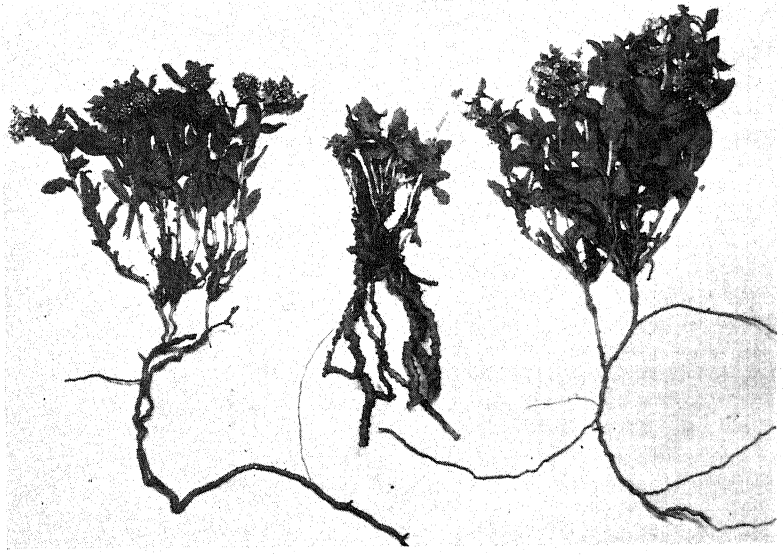


FIGURE 5.—*Lepidium draba:* Outside plants in bud and early blossom stage, with part of root system. Central plant from different plot slower in development; section from root showed five growth rings.

Adventitious roots are also formed from young stem shoots below the ground line. These roots originate from the pericyclic region radially between primary bundles of young stem shoots (pl. 4, *D*).

THE LEAF

Although the external appearance of the leaf has been adequately described by taxonomists, certain external structures should be mentioned here. The pubescence on the leaf surfaces consists of long pointed one-celled trichomes. The trichome cell has a thin covering of the cuticle over its surface. When greatly magnified the hairs appear to have numerous very small elongate stipulate pit markings in their walls. The trichome distribution over the leaf surface was

found to be fairly uniform on the various plants examined. Data taken from 10 leaves from each of 10 scattered plants gave an average of 30 hairs per square millimeter on the lower surface and 25 per square millimeter on the upper surface.

The average number of stomata on the upper and lower surfaces was found to be 273 per square millimeter or 173,355 per square inch on the upper surface of the leaf and 267 per square millimeter on the lower surface. These average numbers are not considered to show a significant difference.

A study of the internal structure of the leaf (pl. 4, *B*) shows it to be very compact. There is marked uniformity of structure in the mesophyll, and intercellular spaces are few. In most of the leaves the mesophyll consists of short palisadelike cells, but in some leaves a poorly developed spongy parenchyma layer was found. Plate 4, *B*, *s*, shows a cross section of a stoma. The transfusion cells around the vascular system of the leaf show best around the large vein bundles.

SUMMARY

The embryo of the dormant seed of hoary cress (*Lepidium draba*) consists of two reflexed cotyledons, a small dome-shaped apical shoot meristem between the cotyledons, and a cylindrical radicle. The provascular system is evident in the axis and cotyledons and consists of undifferentiated procambial cells.

The stele of the root is exarch and diarch.

The root-stem transition is only a short distance below the cotyledons. The protoxylem points are the last of the primary xylem to disappear, and flanking masses of xylem fuse to form the vascular supply to the coytledons. Primary plumular development is independent of that of the primary vascular system of the root and plumule elements are continuous with secondary tissues formed by the cambium in the region of the hypocotyl.

The cortex of the primary root is sloughed off very early; the "cortex" of the older root consists of old phloem.

A secondary wall thickening is deposited in the first layer of cortical cells next to the endodermis of some young primary and secondary roots.

Old roots and the seedling hypocotyl produce adventitious shoots readily.

Less commonly stem shoots form root meristems below the surface of the ground.

Modified stem hairs having characteristics of root hairs were found below the ground line on young stem shoots.

The leaf had a very compact structure suggestive of xerophytic plants and the root cortex of the first 8 inches below the ground contained large intercellular areas somewhat like those of hydrophytic plants.

The leaf stomata are extremely small and very numerous, numbering as many as 173,355 to the square inch.

BLACK RING, A VIRUS DISEASE OF CABBAGE AND OTHER CRUCIFERS[1]

By C. M. TOMPKINS, *assistant plant pathologist*, M. W. GARDNER, *plant pathologist*, and H. REX THOMAS, formerly *graduate assistant in plant pathology, California Agricultural Experiment Station*[2]

INTRODUCTION

A virus disease of cabbage (*Brassica oleracea* L. var. *capitata* L.), designated as black ring, is prevalent mainly during the winter and spring seasons near San Pablo and Colma in the San Francisco Bay section and in the Sacramento, Salinas, and Santa Clara Valleys of California (*18*).[3] Other crucifers of more importance in California, including Brussels sprouts (*B. oleracea* L. var. *gemmifera* DC.) and cauliflower (*B. oleracea* L. var. *botrytis* L.) are also highly susceptible to infection. The results of field and greenhouse studies of this disease, in progress since 1932, are presented in this paper.

REVIEW OF LITERATURE

A mosaic disease of cabbage and other Brassicae, characterized by clearing and necrosis of the veins and yellowing and dwarfing of the plants was briefly recorded by Ogilvie et al. (*8, 9*). Wallflower plants were also found to be susceptible to infection which induced breaking of the flowers.

In 1935, Smith (*12*) described a virus disease of cabbage, Brussels sprouts, and other species of *Brassica*, prevalent near Cambridge, England, which he designated as "ring spot." It seems probable that this disease was identical with that referred to by Ogilvie et al. (*8, 9*). The principal symptoms on cabbage and Brussels sprouts leaves, especially the older ones, consisted of sunken, black, necrotic rings scattered uniformly over the surface. By means of juice inoculations, infection was obtained on cabbage, *Nicotiana glutinosa* L., *N. langsdorffii* Weinm., and *N. tabacum* L. var. White Burley. The insect vector proved to be the green peach aphid (*Myzus persicae* (Sulzer)). Later, Smith (*13, 14*) reported natural infection of wallflower (*Cheiranthus cheiri* L.), annual stock (*Matthiola incana* R. Br. var. *annua* Voss), Brompton stock (*M. incana*), *Arabis* sp., and sweet rocket (*Hesperis matronalis* L.) with the virus of "cabbage mosaic." Apparently the name cabbage mosaic was used in a sense synonymous with ring spot, but this has led to some confusion. The designation of this disease as ring spot is considered unfortunate in view of the earlier use of this term by Weimer (*21*) to describe a ring spot of crucifers caused by *Mycosphaerella brassicicola* (Fr.) Lindau.

Hoggan and Johnson (*6*), in describing a virus disease of crucifers, observed a mild mottling or chlorotic spotting on the leaves of cabbage seedlings inoculated at temperatures of 70° to 80° F. This virus

[1] Received for publication May 18, 1938; issued February 1939.
[2] The writers are indebted to Prof. B. A. Madson and W. W. Mackie for greenhouse space and facilities. Valuable assistance in the greenhouse work was rendered by employees of the Federal Works Progress Administration.
[3] Italic numbers in parentheses refer to Literature Cited, p. 951.

was readily transmissible both mechanically and by means of the cabbage and green peach aphids. No mention was made, however, of necrotic rings as a symptom on this host.

Although the reference by Smith (*12*) is apparently the only one which may directly relate to the black ring disease of cabbage herein discussed, it should be noted for the sake of clarity that certain other crucifer viruses may also cause necrotic lesions on cabbage. These, however, are of a different type. Blank (*1*), workers at the Wisconsin station (*2, 3*), and Walker and Larson (*7, 20*), have discussed a mosaic disease of cabbage, prevalent in Wisconsin, which is characterized by mottling followed by the formation of small, necrotic spots on all of the head leaves. During a recent visit in California, R. H. Larson was shown typical specimens of the black ring disease of cabbage and concluded that it is different from the mosaic disease of cabbage in Wisconsin. Necrotic lesions were produced on cabbage seedlings inoculated with a turnip mosaic virus from New York in recent tests (*16, fig. 5*). These proved to be entirely different from those caused on cabbage plants by the cabbage black ring or the Wisconsin cabbage mosaic virus. Jones [4] reported a mosaic disease of cabbage in Washington but made no mention of necrotic lesions. Recently, through the courtesy of J. F. Adams of Mount Vernon, Wash., the writers received specimens of a virus disease on cabbage in Washington which is similar to, and may be identical with, the black ring virus disease in California. The host range of the Washington cabbage virus closely parallels that of the black ring virus, but the properties have not been studied.

Although Clayton (*4*) found that the rutabaga mosaic virus did not cause necrotic lesions to appear on cabbage leaves, either by natural or experimental means, it is of interest to note that this virus did induce yellow blotches surrounded by necrotic edges on Brussels sprouts leaves (*4, fig. 1, B*).

SYMPTOMS OF THE DISEASE

The symptoms of the disease produced on Winter Colma cabbage by mechanical inoculation in the greenhouse are identical with those observed on different field-grown varieties of cabbage exposed to natural infection. Early symptoms consist of numerous, small, chlorotic, circular to irregular lesions, ranging in size from 0.5 to 1.0 mm in diameter, which are distributed at random between and adjacent to the veins (fig. 1, *A*). When viewed in either reflected or transmitted light, the chlorotic lesions are more prominent and distinct on the upper than on the lower surface of the leaf. Within a few days after the lesions appear there may be a change in color to light viridine yellow [5] accompanied by an increase in size from 1 to 3 mm in diameter. Simultaneously, the chlorotic lesions become more abundant, and there is a gradual transition during which the normal, dark-green color of the leaf is replaced by the chlorotic areas. This gives a marked chlorotic appearance to leaves of infected plants (fig. 1, *B*). Small, dark-green islands now appear against a chlorotic background (fig. 1, *B*). Occasionally, in the early stages of infection, some leaves may show necrotic lesions, chiefly as very small, dark-olive specks and a few large chlorotic areas surrounded by broken to entire, necrotic edges, between and on

[4] JONES, LEON K. OBSERVATIONS ON PLANT DISEASES IN WASHINGTON IN 1936. U. S. Bur. Plant Indus. Plant Disease Reptr. 20: 230–235. 1936.

[5] Color determinations were made with the aid of Ridgway's (*11*) manual.

the veins (fig. 1, *C*). Two to three weeks after the initial appearance of the chlorotic lesions, a necrotic margin develops on many of them, in general less than 0.25 mm in width (fig. 1, *D*), in marked contrast to a normal, healthy cabbage leaf (fig. 1, *E*). When examined with a hand lens, these necrotic rings are seen to be surrounded by a narrow, light-green to light-yellow halo. Not all chlorotic lesions become necrotic. Eventually considerable coalescence of lesions occurs,

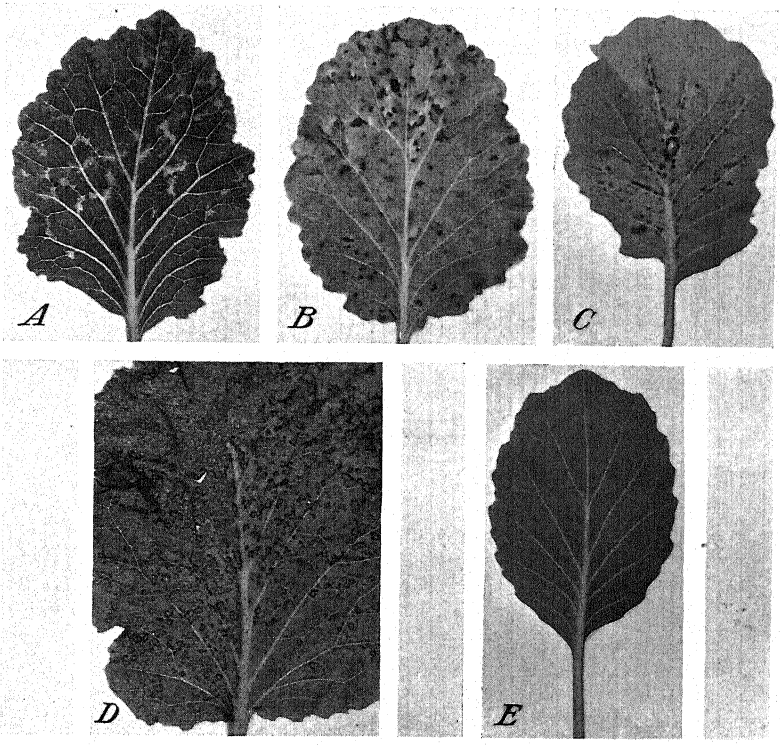


FIGURE 1.—Symptoms produced by the black ring virus on Winter Colma cabbage leaves by mechanical inoculation in the greenhouse at 13° to 19° C.: *A*, Chlorotic lesions on a normal, dark-green background; *B*, dark-green islands on a chlorotic background; *C*, necrotic specks and lesions; *D*, necrotic lesions in late stages of infection; *E*, noninoculated control.

resulting in an irregular, multiple lesion. Where lesions abut directly on a vein or on the midrib, the latter become blackened and shriveled. Eventually the chlorotic to yellow centers of many of the lesions surrounded by necrotic rings become entirely necrotic. The necrotic lesions are generally more numerous and more conspicuous on the lower leaf surface. As the older infected leaves commence to turn yellow with age, the lesions on the upper surface become somewhat more prominent; this is also true of the lesions on the lower surface.

Under natural conditions, symptoms are visible principally on the older, outer head leaves (fig. 2, *A*), although affected plants may be stunted in size. Early and late stages of these necrotic lesions on cabbage leaves, greatly magnified, are shown in figure 2, *D* and *E*. As with greenhouse-infected cabbage plants, the necrotic lesions are most

conspicuous on the under surface of the leaves. When viewed from a distance, a field of infected cabbage plants looks as though it had been scorched by fire. Similar symptoms occur on naturally infected cauliflower leaves (fig. 2, *B*) and on sprouting broccoli (*B. oleracea* var. *botrytis*) (fig. 2, *C*).

In the greenhouse, chlorotic lesions are sometimes larger and more conspicuous on cauliflower than on cabbage leaves (fig. 3, *A*), and in

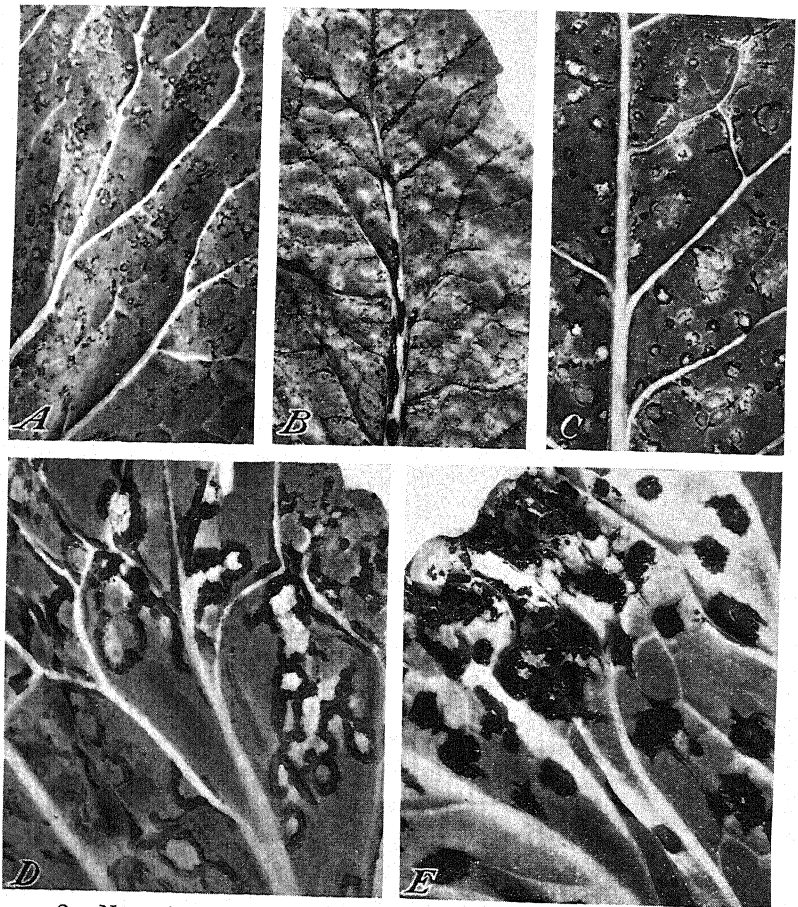


FIGURE 2.—Necrotic lesions produced under natural conditions by the black ring virus: *A*, On cabbage; *B*, on cauliflower; *C* on sprouting broccoli; *D*, single or multiple lesions with green centers on cabbage; *E*, single or multiple lesions, on cabbage, showing complete necrosis.

marked contrast to a healthy cauliflower leaf (fig. 3, *B*). These vary in size and shape and, as with cabbage, some of them may change in color to light viridine yellow. Although necrotic lesions occur on the leaves of naturally infected cauliflower, attempts made in the greenhouse to reproduce this symptom with juice from naturally infected cabbage and cauliflower plants and from the stock culture of the black ring virus maintained on cabbage plants in the greenhouse have all been unsuccessful.

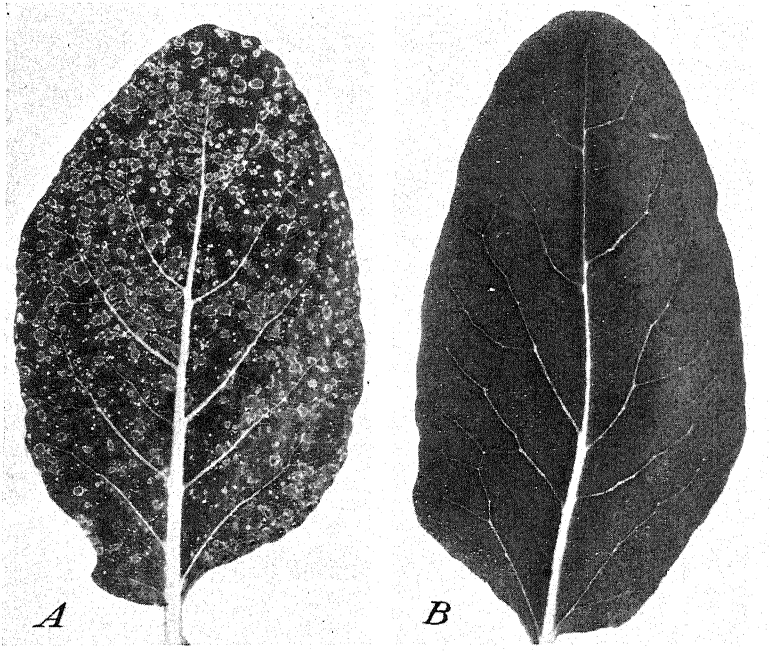


FIGURE 3.—Symptoms produced by the black ring virus by mechanical inoculation in the greenhouse at 13° to 19° C.: *A*, Chlorotic lesions on February cauliflower leaf; *B*, noninoculated control.

TRANSMISSION

The strain of the black ring virus used in studies on transmission, host range, and properties was derived from a diseased cabbage plant collected at San Pablo, Calif., in November 1935. The virus was established on young cabbage plants, variety Winter Colma, in the greenhouse and has been maintained by monthly transfer without any apparent change in virulence. Winter Colma cabbage seedlings were used as the standard test plant for recovery of the virus from infected plants and for property studies.

All inoculations were made in a greenhouse where temperatures ranged from 13° to 19° C. The methods followed were essentially those described in recent papers (*16*, *19*). Mechanical inoculations were made by dusting the leaves with 600-mesh, powdered carborundum (*10*) and lightly rubbing with absorbent cotton dipped in juice from a diseased plant.

The cabbage black ring virus is transmissible to healthy cabbage seedlings by mechanical inoculation only when carborundum (*10*) is used. As a result of numerous tests, the incubation period was determined as ranging from 9 to 21 days.

Since Essig (*5*) found that the cabbage aphid (*Brevicoryne brassicae* (L.)) and the green peach aphid (*Myzus persicae* (Sulzer)) breed naturally on cultivated and wild crucifers in California, it seemed desirable to test these two aphid species under greenhouse conditions.

Following the procedure outlined in a recent paper (*19*), noninfective

cabbage and green peach aphids [6] were fed on recently infected cabbage plants for 24 to 48 hours, after which they were transferred in lots of approximately 20 aphids each to healthy cabbage seedlings. After 24 hours, all plants were sprayed with nicotine sulphate solution. Healthy cabbage seedlings infested with noninfective aphids and noninoculated plants free from aphids served as controls.

Of 20 cabbage plants infested with viruliferous cabbage aphids, 15 showed typical symptoms of the disease in 9 to 16 days. When infective green peach aphids were placed on 20 cabbage plants, 17 plants were infected, the incubation period ranging from 10 to 14 days. The virus was recovered from all infected plants by mechanical inoculation. None of the controls became diseased.

HOST RANGE

Other crucifers in addition to cabbage which are subject to natural infection by the cabbage black ring virus, as proved by mechanical inoculation in the greenhouse with expressed juice from representative field samples, include brussels sprouts, sprouting broccoli, and cauliflower. The chlorotic and necrotic ring symptoms characteristic of infection on cabbage were also observed on all of these hosts.

Numerous commercial varieties of white and red cabbage were tested in the greenhouse by mechanical inoculation to determine their degree of susceptibility. In addition to the variety Winter Colma, previously referred to as the standard test plant, all varieties proved to be highly susceptible. The number of plants of any particular variety to be tested varied from 20 to 200. Symptoms shown by infected plants and the length of the incubation period, irrespective of variety, were in close agreement with those of the variety Winter Colma.

The following varieties of cabbage were tested: White—Allhead Early, All Seasons, Charleston or Large Wakefield, Copenhagen Market (two selections), Danish Ballhead (two selections), Early Drumhead Savoy, Early Dwarf Flat Dutch, Early Jersey Wakefield, Early Winningstadt, Extra Early Express Round, Glory of Enkhuizen, Golden Acre (two selections), Hollander, Improved Globe, Large American Drumhead, Marion Market, Midseason Market, Penn State Ballhead, Perfection Drumhead Savoy, Premium Late Flat Dutch, San Francisco Market, Stone Mason, Succession, Surehead, Volga, Wisconsin All Seasons, and Wisconsin No. 8; red—Mammoth Rock Red (two selections), and Round Red Dutch.

As compared with certain other crucifer viruses which occur in California (*15*, *16*, *19*), the cabbage black ring virus has a more cosmopolitan host range, as shown in table 1. In the family Cruciferae, nine species of Brassica, two species of Matthiola, and seven other genera showed systemic infection. The symptoms obtained on certain crucifers are shown in figures 4 and 5.

Infection was also obtained on plants representing 16 genera and 19 species in 11 additional families, as shown in table 1. Symptoms produced on certain of these hosts are shown in figures 4 and 6.

The virus was recovered from all infected plants with the exception of rhubarb, lambsquarters, sowbane, and spinach. No valid reason

[6] Aphids identified by E. O. Essig, as noninfective cabbage and green peach aphids, were kindly supplied by Dr. H. H. P. Severin and Dr. J. H. Freitag, Division of Entomology and Parasitology, University of California.

can be assigned for failure to recover the virus from these plants on either cabbage or Turkish tobacco.

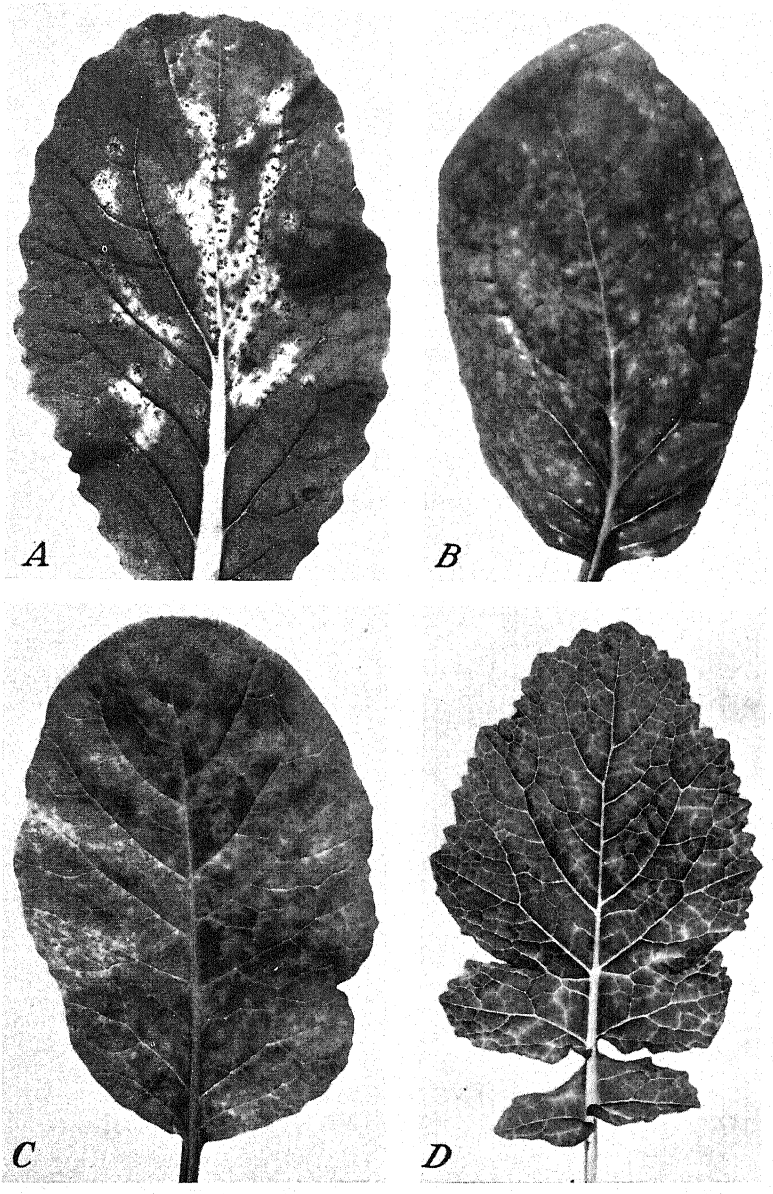


FIGURE 4.—Symptoms produced by the black ring virus on certain host plants after mechanical inoculation in the greenhouse at 13° to 19° C. on: *A*, Chinese cabbage or pe-tsai; *B*, Bloomsdale spinach; *C*, Chinese radish; *D*, Purple Top White Globe turnip.

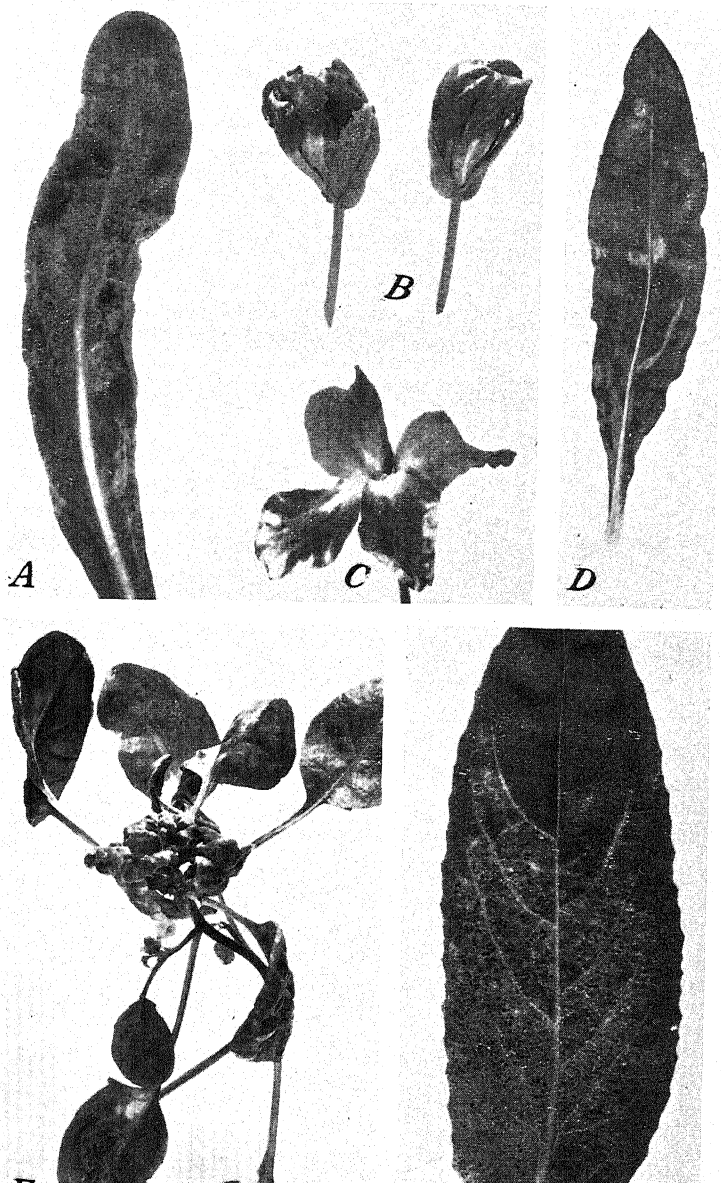


FIGURE 5.—Symptoms produced by the black ring virus on certain ornamental crucifers after mechanical inoculation in the greenhouse at 13° to 19° C.: *A*, *B*, *C*, Leaf, buds, and flower of Fiery Blood Red annual stock; *D*, wallflower; *E*, Virginian stock; *F*, dames violet.

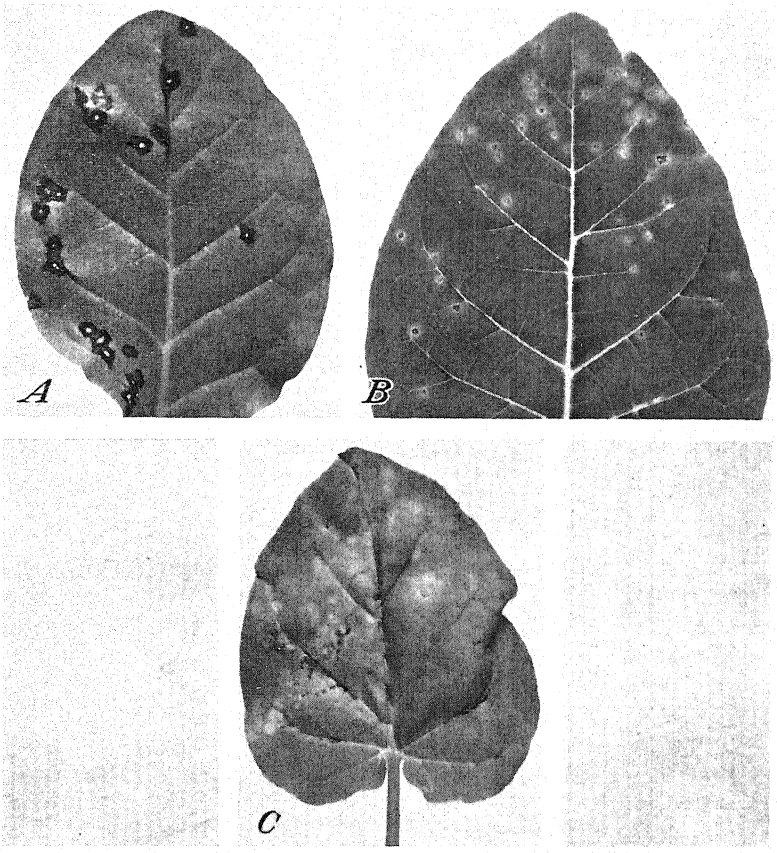


FIGURE 6.—Symptoms produced by the black ring virus on species of *Nicotiana* after mechanical inoculation in the greenhouse at 13° to 19° C.: *A*, Local lesions on *N. tabacum* var. Turkish; *B*, var. White Burley; *C*, *N. glutinosa*.

TABLE 1.—*Plants susceptible to the cabbage black ring virus, as indicated by mechanical inoculation of greenhouse-grown seedlings, and symptoms characteristic of infection*

Family	Species and common name	Symptoms produced
Cruciferae	*Brassica oleracea* L. var. *acephala* DC. (kale).	Systemic. Chlorotic rings. Later, tan, necrotic, irregular-shaped lesions.
	B. oleracea L. var. *gemmifera* DC. (Brussels sprouts).	Systemic. Chlorotic rings which later become necrotic.
	B. oleracea L. var. *botrytis* L. (cauliflower—var. February).	Systemic. Chlorotic rings with marked leaf chlorosis in later stages of infection.
	B. oleracea L. var. *botrytis* L. (asparagus or sprouting broccoli—var. Italian Green Sprouting).	Systemic. Chlorotic rings which later become necrotic.
	B. oleracea L. var. *caulorapa* DC. (kohlrabi—var. Early White Vienna).	Do.
	B. pe-tsai Bailey (pe-tsai)	Do.
	B. napus L. (rape)	Systemic. Chlorotic rings.
	B. campestris L. var. *napobrassica* DC. (rutabaga).	Do.
	B. rapa L. (turnip—var. Purple Top White Globe).	Systemic. Chlorotic rings which later become necrotic. The necrotic lesions are largely confined to the older leaves.
	B. juncea Coss. (leaf mustard)	Systemic. Chlorotic rings.
	B. alba (L.) Boiss. (white mustard)	Systemic. Chlorotic rings confined to inoculated leaves, with vein clearing on new, inner leaves.

TABLE 1.—*Plants susceptible to the cabbage black ring virus, as indicated by mechanical inoculation of greenhouse-grown seedlings, and symptoms characteristic of infection*—Continued

Family	Species and common name	Symptoms produced
Cruciferae	*B. adpressa* Boiss	Systemic. Chlorotic rings.
	B. arvensis (L.) Ktze. (charlock)	Systemic. Chlorotic rings which later become necrotic. Infection caused early death of plants.
	Capsella bursa-pastoris (L.) Medic. (Shepherdspurse).	Systemic. Chlorotic rings and downward curling of leaf.
	Cheiranthus cheiri L. (wallflower)	Systemic. Chlorotic rings, with some fusion.
	Matthiola bicornis DC. (evening scented stock).	Systemic. A bleaching type of mottling.
	M. incana R. Br. (Brompton stock)	Systemic. Chlorotic rings, coarse vein clearing, and some leaf distortion.
	M. incana R. Br. var. *annua* Voss (annual stock).	Systemic mottling causing rapid bleaching of leaf and leaving small, dark-green islands. Breaking of flowers occurred.
	Hesperis matronalis L. (dames violet)	Systemic. Vein clearing of young, inner leaves followed by development of chlorotic and necrotic lesions.
	Malcomia maritima R. Br. (Virginian stock).	Systemic. Mottling and rosetting of new growth.
	Radicula nasturtium-aquaticum Brit. & Rend. (water cress).	Systemic. Chlorotic rings.
	Lunaria annua L. (honesty)	Systemic. Mottling and bronzing of leaf, with vein necrosis.
	Raphanus sativus L. var. *longipinnatus* Bailey (Chinese radish).	Systemic. Chlorotic rings.
Begoniaceae	*Begonia semperflorens* Link and Otto (fibrous-rooted begonia).	Do.
Boraginaceae	*Myosotis alpestris* Schmidt (forget-me-not).	Systemic. Chlorotic and necrotic lesions on leaves and petioles.
Caryophyllaceae	*Stellaria media* (L.) Cyrill (chickweed)	Systemic. Chlorotic rings and mottling.
Chenopodiaceae	*Chenopodium album* L. (lambsquarters or white pigweed).	Systemic. Chlorotic to yellow rings 1/16 inch in diameter which later enlarge to 3/16 inch. The pale to yellow centers surrounded by narrow, chlorotic band or halo.
	C. murale L. (sowbane or nettle-leaf goosefoot).	Systemic. Chlorotic rings which later turn yellow with a green edge.
	Spinacia oleracea L. (spinach—var. Bloomsdale)	Systemic. Chlorotic rings which later turn a bright yellow and fuse, giving the leaf a yellowed appearance.
Compositae	*Chrysanthemum coronarium* L. (annual marguerite).	Systemic. Vein clearing.
	Dimorphotheca aurantiaca DC. (winter Cape-marigold).	Systemic. Chlorotic rings become necrotic around the edges.
	Senecio cruentus DC. (hybrid cineraria).	Systemic. Vein necrosis and chlorosis of young, inner leaves.
	Zinnia elegan Jacq. (zinnia or youth-and-old-age).	Systemic. Vein clearing.
Dipsacaceae	*Scabiosa atropurpurea* L. (mourning bride or pincushion flower).	Systemic. Vein clearing.
Papaveraceae	*Papaver nudicaule* L. (Iceland poppy)	Systemic. Leaves mottled and containing necrotic lesions surrounded by chlorotic halos. Necrotic streaks on petioles.
Polygonaceae	*Rheum rhaponticum* L. (rhubarb)	Systemic. Chlorotic rings.
Resedaceae	*Reseda odorata* L. (mignonette)	Do.
Solanaceae	*Nicotiana glutinosa* L	Systemic. Chlorotic rings at first, followed by necrotic lesions which appeared on older, outer leaves only.
	N. langsdorffii Weinm	Systemic. Mottling and tan, necrotic lesions.
	N. tabacum L. (tobacco—var. Turkish)	Local, tan necrotic lesions on inoculated leaves only. Not systemic.
	——var. White Burley	Chlorotic rings with necrotic centers on inoculated leaves only. Not systemic.
	Petunia hybrida Hort. (petunia)	Systemic. Chlorotic rings. Curling and puckering of leaves and stunting of plant.
Verbenaceae	*Verbena hybrida* Voss (garden verbena)	Systemic. Chlorotic rings with necrosis of veins and midrib.

No infection was obtained on other plants representing 26 families, 49 genera, and 53 species, as follows:

Boraginaceae:
Alkanet (*Anchusa officinalis* L.)
Common heliotrope (*Heliotropium peruvianum* L.)

Campanulaceae: Canterbury-bells (*Campanula medium* L.)
Caryophyllaceae:
- Sweet-william (*Dianthus barbatus* L.)
- Babysbreath (*Gypsophila paniculata* L.)

Chenopodiaceae:
- Sugar beet (*Beta vulgaris* L.)
- Swiss chard (*B. vulgaris* var. *cicla* Moq.)

Compositae:
- Head lettuce (*Lactuca sativa* L. var. *capitata* Hort.) var. New York and Tom Thumb
- Dandelion (*Taraxacum officinale* Weber)
- Transvaal daisy (*Gerbera jamesonii* Hook. var. *transvaalensis* Hort.)
- Shasta daisy (*Chrysanthemum maximum* Ram.)
- *Dahlia* sp.
- China-aster (*Callistephus chinensis* Nees) var. Giant Branching White, wilt resistant
- English daisy (*Bellis perennis* L.)
- French marigold (*Tagetes patula* L.)
- *Gaillardia pulchella* Foug. var. *picta* Gray

Convolvulaceae: Morning-glory (*Ipomoea purpurea* Roth)
Cruciferae:
- *Brassica integrifolia* O. E. Schulz var. *chevalieri* R. Porteres
- Rockcress (*Arabis albida* Stev.)
- Goldentuft (*Alyssum saxatile* L.)
- Radish (*Raphanus sativus* L.) var. White Icicle

Cucurbitaceae: Cucumber (*Cucumis sativus* L.)
Euphorbiaceae: Castor-bean (*Ricinus communis* L.)
Geraniaceae: Storksbill (*Pelargonium zonale* Willd.)
Gramineae:
- Corn (*Zea mays* L.) var. Golden Bantam
- Oat (*Avena sativa* L.)

Labiatae:
- Flowering sage (*Salvia farinacea* Benth.)
- Bugleweed (*Ajuga reptans* L. var. *rubra* Hort.)

Leguminosae:
- Garden pea (*Pisum sativum* L.) var. Alderman
- Broadbean (*Vicia faba* L.)
- Alfalfa (*Medicago sativa* L.) var. Arabian, Baltic, California Common, Cossack, Grimm, Hardistan, Hemet, and Modoc

Lobeliaceae: Lobelia (*Lobelia hybrida* Hort.)
Malvaceae:
- Cheeseweed (*Malva parviflora* L.)
- Hollyhock (*Althaea rosea* Cav.)

Onagraceae:
- *Clarkia elegans* Dougl.
- *Godetia grandiflora* Lindl.

Papaveraceae: Oriental poppy (*Papaver orientale* L.)
Plumbaginaceae: Everlasting or sea-lavender (*Statice latifolium* Kuntze)
Ranunculaceae:
- Rocket larkspur (*Delphinium ajacis* L.)
- Hybrid delphinium (*D. cultorum* Voss)

Rosacea: *Geum chiloense* Balb.
Scrophulariaceae:
- Snapdragon (*Antirrhinum majus* L.)
- Pentstemon or beardtongue (*Pentstemon barbatus* Nutt.)

Solanaceae:
- *Solanum aviculare* Forst.
- Potato (*S. tuberosum* L.)
- Eggplant (*S. melongena* L. var. *esculentum* Nees)
- Tomato (*Lycopersicum esculentum* Mill. var. *vulgare* Bailey) var. Early Santa Clara Canner
- Currant tomato (*L. pimpinellifolium* Dunal)
- *Nicotiana rustica* L. var. *humulis* Schrank
- Jimsonweed (*Datura stramonium* L.)

Tropaeolaceae: Garden nasturtium (*Tropaeolum majus* L.)
Umbelliferae: Celery (*Apium graveolens* L.) var. Golden Self Blanching
Valerianaceae: Garden heliotrope (*Valeriana officinalis* L.)
Violaceae: Pansy (*Viola tricolor* L.)

PROPERTIES OF THE VIRUS

Three properties of the black ring virus of cabbage were studied, as shown in table 2. The virus was infectious after being stored for 48 hours at 22° C. but was inactivated after 72 hours. Of 25 cabbage plants inoculated with virus extract heated for 10 minutes at 57°, only 1 plant became infected. No infection resulted from inoculations with virus extract heated at 59°. It is concluded, therefore, that the inactivation temperature lies between 57° and 59°. The virus has a dilution tolerance of approximately 1 to 700, beyond which infection did not occur.

In their studies of the properties of a cabbage mosaic virus in Wisconsin, Larson and Walker (7) reported inactivation at 55° C. for a 10-minute exposure. A dilution of 1 to 1,000 and storage for 2 days at 20° to 22° did not inactivate the virus.

TABLE 2.—*Longevity in vitro, inactivation temperature, and tolerance to dilution of the cabbage black ring virus*

[Five trials made with 25 plants each in all instances]

LONGEVITY IN VITRO, 22° C.

Aged (hours)	Plants infected	Aged (hours)	Plants infected	Aged (hours)	Plants infected	Aged (hours)	Plants infected
	Number		*Number*		*Number*		*Number*
0	25	8	13	48	4	96	0
4	20	24	19	72	0	120	0

INACTIVATION TEMPERATURE (10 MINUTES)

Temperature (°C.)	Plants infected	Temperature (°C.)	Plants infected	Temperature (°C.)	Plants infected	Temperature (°C.)	Plants infected
	Number		*Number*		*Number*		*Number*
Not heated	25	55	15	59	0	65	0
50	23	57	1	60	0	70	0

TOLERANCE TO DILUTION

Dilution	Plants infected	Dilution	Plants infected	Dilution	Plants infected	Dilution	Plants infected
	Number		*Number*		*Number*		*Number*
0	20	1:50	4	1:500	1	1:2,000	0
1:10	14	1:100	5	1:700	3		
1:25	8	1:200	4	1:1,000	0		

DESCRIPTION OF THE BLACK RING VIRUS OF CABBAGE

Transmissible by mechanical inoculation with expressed juice and powdered carborundum at temperatures of 13° to 19° C. Transmitted in greenhouse tests by means of cabbage aphid (*Brevicoryne brassicae* (L.)) and green peach aphid (*Myzus persicae* (Sulzer)). Incubation period 9 to 21 days. Resistance to aging in vitro between 48 and 72 hours. Inactivation temperature at or near 59° C. for 10-minute exposure. Tolerance to dilution approximately 1 to 700. Cabbage (*Brassica oleracea* L. var. *capitata* L.) and numerous other vegetable and ornamental crucifers susceptible. On cabbage, symptoms consist of numerous chlorotic rings, some becoming necrotic with age. Occasionally necrotic streaking of veins. In the field, symptoms are conspicuous only on older leaves.

COMPARISON OF THE BLACK RING VIRUS WITH CERTAIN OTHER CRUCIFER VIRUSES

The viruses of cauliflower mosaic (*16*), Chinese cabbage mosaic (*19*) turnip mosaic (*17*), mild and severe annual stock mosaic (*15*)[7], and radish mosaic [7] were tested simultaneously with the black ring virus in the greenhouse in parallel series of inoculations to various plants. For comparative purposes, the more important symptom differences on certain differential hosts are presented.

On Winter Colma cabbage, the cauliflower mosaic virus (*16, fig. 3, C*) causes vein clearing; the Chinese cabbage mosaic virus (*19, fig. 2, C*), coarse, yellowish, vein banding; the turnip mosaic virus (*16, fig. 5, A; 17, fig. 2*), chlorotic rings with necrotic edges; and the radish mosaic virus, large chlorotic rings, some of which later become necrotic, but differ in color, size, and shape from those produced by the black ring virus. No infection of cabbage was obtained with either the mild or the severe mosaic virus of annual stock.

On February cauliflower, the cauliflower mosaic virus caused vein clearing, followed by vein banding and necrotic spotting (*16, fig. 1, A, B, C*); the Chinese cabbage mosaic virus, scattered chlorotic rings with dark-green centers (*19, fig. 2, B*); the turnip mosaic virus, pale-green to yellow lesions (*17, fig. 5, B*); and the radish mosaic virus, diffuse chlorotic lesions. No infection of cauliflower was obtained with either the mild or the severe mosaic virus of annual stock.

On Chinese cabbage, or pe-tsai, the black ring virus, as already shown, caused chlorotic rings, some of which later became necrotic. The cauliflower mosaic virus (*16*) caused vein clearing; the Chinese cabbage mosaic virus (*19, fig. 1, A, B, C*), vein clearing and mottling; the turnip mosaic virus (*17*), coarse yellow vein banding and leaf distortion; the mild mosaic virus of annual stock, a fine to medium type of mottling; and the radish mosaic virus, chlorotic and necrotic lesions and vein necrosis on inoculated leaves only. The severe mosaic virus of annual stock did not cause infection.

On Purple Top White Globe turnip, the black ring virus induced chlorotic rings, some of which were necrotic. The cauliflower mosaic virus (*16*) caused vein clearing; the Chinese cabbage mosaic virus (*19*), a fine type of vein clearing and mottling; the turnip mosaic virus, vein clearing followed by mottling with raised islands, crinkling, and stunting of plants; the mild and severe mosaic viruses of annual stock, a fine to medium type of mottling; and the radish mosaic virus, mottling with severe distortion of leaves.

Numerous unsuccessful attempts were made to infect White Icicle radish with the black ring virus. Paralleling this is the fact that the turnip (*17*) and severe annual stock mosaic viruses do not infect radish. On the other hand, the cauliflower mosaic virus (*16*) caused vein clearing; the Chinese cabbage mosaic virus (*19, fig. 2, C*), vein clearing and mottling; the mild mosaic virus of annual stock, a fine to medium type of mottling; and the radish mosaic virus, coarse mottling and raised dark-green islands on a chlorotic to yellow background.

The black ring virus caused mottling of the leaves and flower breaking on Fiery Blood Red annual stock; the cauliflower mosaic virus (*16, fig. 4, A, B, C*), coarse vein clearing but no flower breaking; the Chinese cabbage mosaic virus (*19*), mottling of leaves and flower

[7] The mild and severe mosaic diseases of annual stock and a mosaic disease of radish are fully described in papers now in press.

breaking; the turnip mosaic virus (*17*), coarse vein clearing, diffuse mottling, and flower breaking; the mild annual stock mosaic virus, a fine type of mottling and flower breaking; and the severe mosaic virus of annual stock, very coarse, conspicuous leaf mottling and flower breaking. No infection was obtained with the radish mosaic virus.

SUMMARY

A virus disease of cabbage, cauliflower, and other cultivated crucifers, designated as black ring, is described in this paper.

The disease occurs chiefly in the cool, coastal valleys of California during the winter months and is uncommon in the summer.

The disease causes marked damage to the older, outer leaves. It is characterized by chlorotic lesions, many of which become partially or entirely necrotic with age. The symptoms are most conspicuous on the under surface of the leaf.

Under greenhouse conditions, the black ring virus has been transmitted to healthy cabbage plants by means of the cabbage and green peach aphids. It is also readily transmissible by juice inoculations with powdered carborundum.

The inactivation temperature of the virus lies between 57° and 59° C. The virus withstands aging in vitro at 22° C. for 48 hours, and has a tolerance to dilution of 1 to 700.

All tested varieties of cabbage proved susceptible.

In the family Cruciferae, systemic infection was obtained on kale, Brussels sprouts, sprouting broccoli, kohlrabi, turnip, pe-tsai, charlock, dames violet, cauliflower, rape, rutabaga, leaf or Chinese mustard, white mustard, *Brassica adpressa*, shepherdspurse, wallflower, Brompton stock, water cress, Chinese radish, evening scented stock, annual stock, and honesty.

Infection was also obtained on plants representing 11 additional families, as follows: Rhubarb, lambsquarters, sowbane, spinach, chickweed, mignonette, fibrous-rooted begonia, garden verbena, petunia, Iceland poppy, *Nicotiana glutinosa*, *N. langsdorffii*, *N. tabacum*, mourning bride, annual marguerite, zinnia, winter Cape-marigold, cineraria, and forget-me-not.

LITERATURE CITED

(1) Blank, L. M.
1935. A mosaic on cabbage in Wisconsin. (Abstract) Phytopathology 25: 6.

(2) Clark, Noble, comp.
1935. Mosaic found to affect cabbage. Wis. Agr. Expt. Sta. Bull. 430: 25.

(3) ———and Hoveland, Niemen, comp.
1937. Cabbage mosaic. Wis. Agr. Expt. Sta. Bull. 438: 113–114, illus.

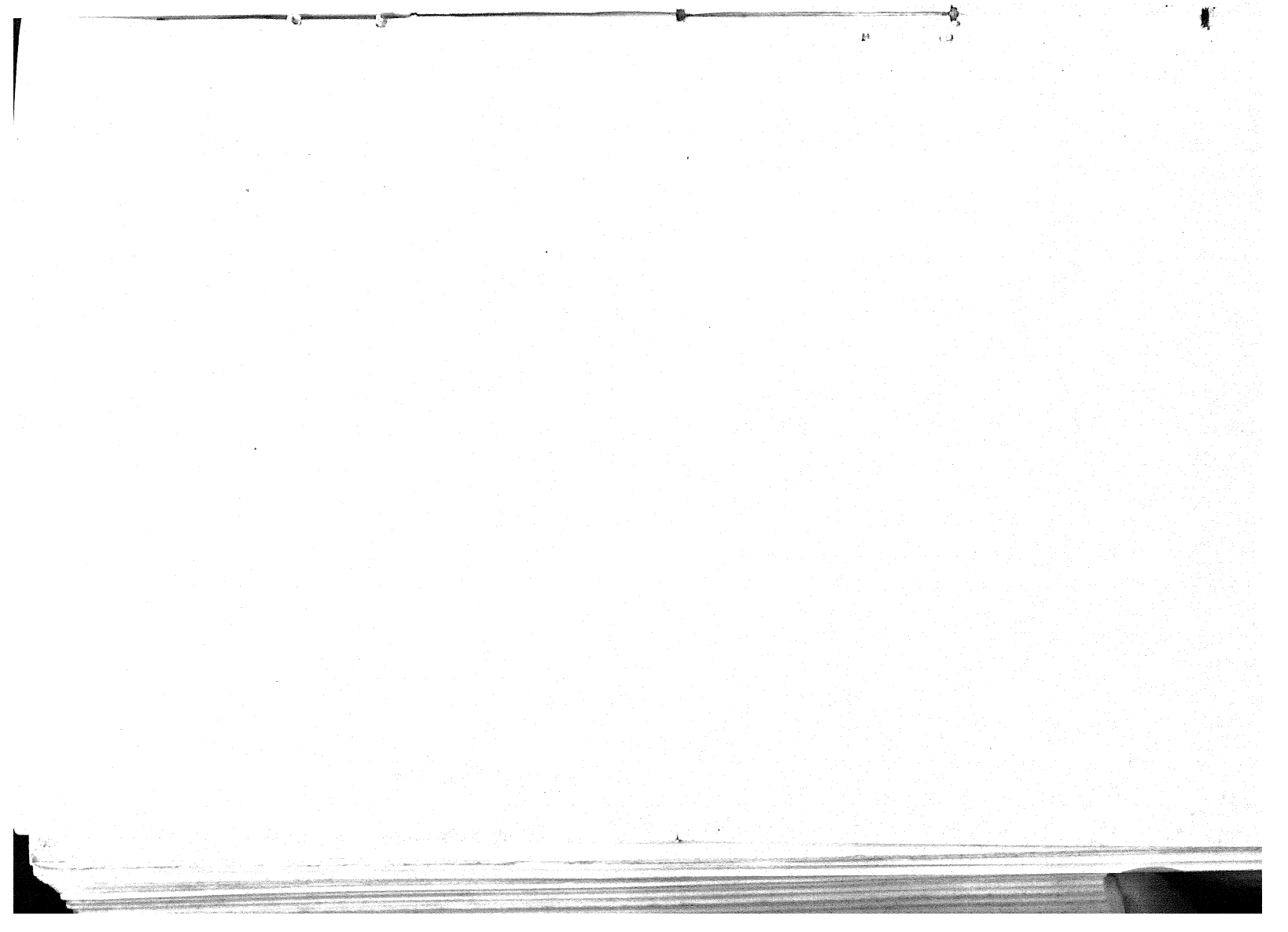
(4) Clayton, E. E.
1930. A study of the mosaic disease of crucifers. Jour. Agr. Research 40: 263–270, illus.

(5) Essig, E. O.
1929. Insects of western North America . . . 1,035 pp., illus. New York.

(6) Hoggan, Isme A., and Johnson, James.
1935. A virus of crucifers and other hosts. Phytopathology 25: 640–644, illus.

(7) Larson, R. H., and Walker, J. C.
1938. Properties and host range of a cabbage mosaic virus. (Abstract) Phytopathology 28: 13.

(8) OGILVIE, L., and HICKMAN, C. J.
1936. PROGRESS REPORT ON VEGETABLE DISEASES.—VIII. Ann. Rept. Agr. and Hort. Research Sta., Long Ashton, Bristol. 1936: 139–148.
(9) ——— MULLIGAN, B. O., and BRIAN, P. W.
1934. PROGRESS REPORT ON VEGETABLE DISEASES.—VI. Ann. Rept. Agr. and Hort. Research Sta., Long Ashton, Bristol, 1934: 175–190, illus.
(10) RAWLINS, T. E., and TOMPKINS, C. M.
1936. STUDIES ON THE EFFECT OF CARBORUNDUM AS AN ABRASIVE IN PLANT VIRUS INOCULATIONS. Phytopathology 26: 578–587, illus.
(11) RIDGWAY, ROBERT.
1912. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 pp., illus. Washington, D. C.
(12) SMITH, KENNETH M.
1935. A VIRUS OF CULTIVATED CRUCIFERS. Ann. Appl. Biol. 22: 239–242, illus.
(13) ———
1935. COLOUR CHANGES IN WALLFLOWERS AND STOCKS. Gard. Chron. 98: 112, illus.
(14) ———
1936. THE VIRUS DISEASES OF GLASSHOUSE AND GARDEN PLANTS. Sci. Hort. 4: 126–140, illus.
(15) TOMPKINS, C. M.
1934. BREAKING IN STOCK (MATHIOLA INCANA), A VIROSIS. (Abstract) Phytopathology 24: 1137.
(16) ———
1937. A TRANSMISSIBLE MOSAIC DISEASE OF CAULIFLOWER. Jour. Agr. Research 55: 33–46, illus.
(17) ———
1938. A MOSAIC DISEASE OF TURNIP. Jour. Agr. Research 57 (8) 589–602, illus.
(18) ——— GARDNER, M. W., and THOMAS, H. REX.
1937. BLACK RING, A VIROSIS OF CABBAGE AND OTHER CRUCIFERS. (Abstract) Phytopathology 27: 955–956.
(19) ——— and THOMAS, H. REX.
1938. A MOSAIC DISEASE OF CHINESE CABBAGE. Jour. Agr. Research 56: 541–551, illus.
(20) WALKER, J. C., and LARSON, R. H.
1937. INCREASING IMPORTANCE OF CABBAGE MOSAIC. (Abstract) Phytopathology 27: 142.
(21) WEIMER, J. L.
1926. RINGSPOT OF CRUCIFERS CAUSED BY MYCOSPHAERELLA BRASSICICOLA (FR.) LINDAU. Jour. Agr. Research 32: 97–132, illus.



SOIL-FERTILITY STANDARDS FOR GROWING NORTHERN CONIFERS IN FOREST NURSERIES[1]

By S. A. WILDE [2]

Associate professor of soils, Wisconsin Agricultural Experiment Station, and senior assistant forester, Wisconsin Conservation Department

INTRODUCTION

In nursery management, the state of soil fertility is one of the most important factors that affect the development of seedlings. A deficiency of any of the essential nutrients or an unbalanced ratio has a far-reaching influence on the entire process of metabolism. The ill effects of malnutrition are reflected in abnormal size and color of leaves, inadequate root systems, unbalanced top-root ratio, thin cell walls and porous structure of wood tissue, underdeveloped nuclei in the parenchymatic cells, and other anatomical and physiological abnormalities (*4, 5, 8, 9*).[3] Ultimately, all of these ailments result in a decreased resistance of seedlings to drought, frost, and infectious diseases.

CONTENT OF NUTRIENTS AND ASSOCIATED CONDITIONS IN VIRGIN FOREST SOILS

Recently cleared but not burned-over forest land, which previously supported a mature or nearly mature stand of satisfactory growth, when used as a nursery site, always produces at least one crop of vigorous seedlings of the same species that originally grew on the area. This is common knowledge acquired from many years of experience, particularly with temporary nurseries in Europe. Consequently, the nutrient content of productive forest soils is a satisfactory criterion for establishing soil-fertility standards in forest nurseries. Based on this fact, a detailed study of the chemical properties of soils supporting normally developed stands of jack pine (*Pinus banksiana* Lamb.), red pine (*P. resinosa* Ait.), northern white pine (*P. strobus* L.), white spruce (*Picea glauca* (Moench) Voss), and balsam fir (*Abies balsamea* (L.) Mill.), and mixed stands of northern hardwoods (*Acer saccharum* Marsh., *Tilia americana* L., *Betula lutea* Michx. f., etc.,) has been carried out. The minimum age of the jack pine stands was 25 years and that of the other species 40 years.

About 500 soil samples were collected from the following areas: Chippewa and Superior National Forests and Cloquet Experimental Forest in Minnesota; Chequamegon and Nicolet National Forests, the Wisconsin River Valley from Wisconsin Dells to Stevens Point, and Brule, Northern, and American Legion State Forests in Wisconsin; Ottawa, Hiawatha, and Huron National Forests, Manistee pur-

[1] Received for publication May 31, 1938. Issued February 1939. Contribution from the Soils Department, Wisconsin Agricultural Experiment Station, in cooperation with the Wisconsin Department of Conservation. The work was supported by Works Progress Administration funds.

[2] Credit is due S. F. Buran, senior chemist, who was engaged in laboratory analyses connected with this study. H. M. Galloway and J. G. Cady assisted in field work, statistical treatment of the results, and preparation of the manuscript.

[3] Italic numbers in parentheses refer to Literature Cited, p. 951

chase unit, and the Higgings Lake region in Michigan; Hoosier purchase unit in Indiana; Gardner purchase unit in Missouri; and the Wayne purchase unit in Ohio.

The results of analyses for pH value (*10*), silt and clay fractions (*2*), base exchange capacity (*3*), total nitrogen (*1*), nitrates (*6*), ammonia (*7*), available phosphorus (*11*), available potassium (*12*), replaceable calcium, and replaceable magnesium (*3*) are given in tables 1, 2, and 3.

TABLE 1.—*Silt, clay, and organic-matter content and base-exchange properties of soils of various forest sites*

Site	Silt, 0.05–0.005 mm	Clay, less than 0.005 mm	Total fine separates	Organic matter [1]	Base-exchange capacity per 100 g	Total replaceable bases per 100 g	Base saturation
	Percent	*Percent*	*Percent*	*Percent*	*Milli-equivalents*	*Milli-equivalents*	*Percent*
Barrens	3.8±0.6	1.1±0.1	4.9	1.93	2.18±0.2	1.5	68.8
Jack pine	7.4± .4	2.0± .2	9.4	3.76	4.30± .2	2.6	60.5
Red pine	10.6±1.3	3.4± .9	14.0	4.68	8.26± .4	4.3	52.0
White pine	12.0± .9	6.4± .8	18.4	5.25	9.60± .5	6.7	69.8
White spruce	25.3±2.4	28.6±1.5	53.9	7.19	15.10± .9	8.5	56.3
Hardwoods	28.8±2.1	24.4±1.8	53.2	6.85	16.30± .5	12.9	79.1

[1] Approximate figures for "loss on ignition," arrived at largely on the basis of total nitrogen determinations.

TABLE 2.—*Content of nitrate and ammonia nitrogen and its relation to the content of total nitrogen in soils of different forest sites*

Site	Medians for—		Arithmetic means of—			Total N	Relation of available N to total N	Approximate level of available N per acre
	Nitrate N	Ammonia N	Nitrate N	Ammonia N	Total available N			
	P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*Percent*	*Pounds*
Barrens	Tr.	Tr.	0.8	2.2	3.0	430	0.69	9
Jack pine	Tr.	5	2.3	6.0	8.3	1,040	.79	20
Red pine	Tr.	11	1.6	9.9	11.5	1,190	.96	29
White pine	Tr.	10	2.1	11.8	13.9	1,420	.98	35
White spruce	2	24	2.9	19.0	21.9	2,570	.85	44
Hardwoods	10	Tr.	11.5	5.7	17.2	2,210	.78	35

TABLE 3.—*Reaction and content of nutrients in soils of different forest sites*

Site	Reaction	Total N	Available P_2O_5 per acre	Available K_2O per acre	Replaceable Ca per 100 g	Replaceable Mg per 100 g
	pH	*Percent*	*Pounds*		*Milli-equivalents*	*Milli-equivalents*
Barrens	6.30±0.12	0.043±0.005	25.8± 2.5	66.9± 9.5	1.1±0.2	0.3±0.1
Jack pine	5.60± .07	.104± .003	37.5± 6.3	92.0± 7.24	1.9± .1	.5± .1
Red pine	5.33± .11	.119± .004	43.9± 2.7	152.8±10.2	3.2± .2	.9± .1
White pine	5.40± .10	.142± .005	82.8± 9.6	192.0±11.0	5.0± .4	1.4± .2
White spruce	5.19± .09	.257± .010	98.3± 9.9	287.4±13.3	6.1± .7	2.1± .2
Hardwoods	6.07± .07	.221± .015	175.8±13.1	301.7±20.6	10.3± .8	2.4± .1

The mean values presented in these tables were derived from 30 to 40 single figures expressing the fertility of the upper 8-inch layer of the

soil profile. These figures for individual soil profiles were obtained by the analysis of soil samples collected from the separate soil horizons, such as litter (A_{00}), duff (A_0), humic layer (A_1), and leached podsolic layer (A_2). The average thickness of each horizon, its volume weight, and its content of nutrients were considered in computing the total fertility of each 8-inch layer. An example will illustrate the method employed. A layer of litter and duff 2 inches in thickness contains 300 p. p. m. of available phosphorus pentoxide, whereas the 6-inch layer of mineral soil underlying the duff contains 40 p. p. m. of phosphorus pentoxide. The weight per acre of 2-inch duff and 6-inch loam layers is 240,000 and 2,000,000 pounds, respectively. Thus, the content of phosphorus pentoxide in the duff layer is $240{,}000 \times 0.0003$, or 72 pounds, whereas the content of the same constituent in mineral soil is $2{,}000{,}000 \times 0.00004$, or 80 pounds. Hence, the total amount of available P_2O_5 in a soil layer 8 inches deep is $72+80$ or 152 pounds per acre, or per 2,240,000 pounds of soil. This corresponds to $152 \times 1{,}000{,}000 \div 2{,}240{,}000$, or 67 p. p. m. This is the content of phosphorus pentoxide which will be found in the top 8 inches of nursery soil after the analyzed layers have been mixed by plowing and disking or by rototilling.

The analysis of soil was not extended beyond a depth of 8 inches since the soil at greater depths does not affect the nutrition of young seedlings in the nursery. The standard deviations for establishing standard errors were calculated by dividing the sum of squared deviations by $n-1$. The data for available nitrogen given in table 2 cannot be regarded as having more than a relative value, since the amounts of ammonia and nitrates vary greatly with the season and other conditions. Because of this variability, medians, as well as arithmetic means, are reported for these two factors.

Table 4 presents in round numbers the approximate desirable levels or ranges of different soil factors, which, with the qualifications discussed below, may serve as standards in the maintenance of nursery soil fertility. The use of these standards has been tested with satisfactory results in about 30 Federal, State, and private forest nurseries located in Wisconsin and several neighboring States.

The barren soils were analyzed simply in order to determine the lowest level of soil fertility. The study of hardwood soils was not confined to any particular species, and requires additional analyses. Consequently, no data for these two sites are given in table 4.

TABLE 4.—*Standards of fertility for nursery blocks raising different species of trees*

Species	Reaction	Base-exchange capacity per 100 g	Total N	Approximate level of available N per acre	Available P_2O_5 per acre	Available K_2O per acre	Replaceable Ca per acre	Replaceable Mg per acre
	pH	*Milliequivilents*	*Percent*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
Jack pine	5.6	5.0	0.10	20	40	100	1,000	150
Red pine	5.4	8.0	.12	30	50	150	1,500	300
White pine	5.4	10.0	.14	35	80	200	2,500	450
White spruce	5.2	15.0	.25	45	100	275	3,000	600

The typological investigations in Europe, as well as greenhouse studies at this Station, have indicated that the standards established for red pine and white spruce may be safely applied to Scotch pine (*Pinus sylvestris* L.) and Norway spruce (*Picea excelsa* Link).

PRINCIPLES OF NURSERY SOIL IMPROVEMENT

Ordinarily, soil improvement involves a period of several years and follows a definitely outlined program. Only a few nurseries may be brought to a satisfactory level of fertility by means of a single application of fertilizer and absorbing materials. The time element is particularly important when a nursery soil has a low content of exchange material and total nitrogen, or considerable deviation of pH value from the desired state. In many instances even the correction of potassium and phosphorus deficiency may require a period of several years.

In outlining a soil-improvement program it is important to keep two conditions in mind. The first is the undesirability of introducing large quantities of raw organic remains and mineral fertilizers, for such introduction may bring about a deficiency of available nitrogen and a toxicity of soil solution due to a high concentration of salts. On the other hand, undue delay in attaining full productive capacity of the nursery soil may result in financial losses caused by waste of unabsorbed fertilizers and may lead to production of underdeveloped stock. Therefore, expert judgment is necessary in determining the time needed to complete the soil-building program.

It is important to realize that the nutrient requirements of seedlings cannot be satisfied by a purely arithmetic balance between the mineral constituents of fertilizers and their consumption by stock, but only through complex physiochemical and biological processes. The growth of seedlings in a soil containing unabsorbed fertilizer salts is different from the growth in medium in which the salts have become incorporated into organic matter by means of chemical and biological reactions. In the first case, the nutrient medium is characterized by equal amounts of cations and anions, great periodic changes in concentration of soil solution due to fluctuations of soil moisture, and a number of other conditions which are not favorable to the normal nutrition of forest trees. In the second case, most of the nutrients are present as complex organic compounds, and the absorbed cations predominate in the soil since the anions are largely eliminated by leaching. It is evident, therefore, that the soil-building process is not completed merely by the addition of a certain amount of peat and mineral fertilizers, but only when these materials have undergone a certain period of "composting" and have become incorporated with the soil through microbiological activity and exchange reactions.

GENERAL DIRECTIONS FOR ADJUSTMENT OF SOIL CONDITIONS

The adjustment of pH value of soil has recently received considerable attention in both agricultural and phytopathological literature. The correction of colloid content, base exchange capacity, and total nitrogen has been previously outlined[4] (*13*, *16*).

[4] WILDE, S. A. ADJUSTMENT OF SOIL FERTILITY IN CONIFEROUS NURSERIES. Wis. State Conserv. Dept. in Coop. with Wis. Agr. Col. Tech. Notes 19, 9 pp. 1937. [Mimeographed.]

Under favorable conditions of nursery soil, the activity of microorganisms provides a sufficient amount of available nitrogen by converting protein compounds into ammonia and nitrates. The usual content of nitrate and ammonia nitrogen together in a nursery soil amounts to about 1 percent of the total nitrogen [4] (*9*), i. e., about 40 pounds per acre, if nursery soil contains 0.2 percent of total nitrogen. Under such well-balanced conditions there is no need to apply available nitrogen in the form of commercial fertilizers. In many instances, however, the amount of available nitrogen released by soil organisms may not be sufficient for seedling growth. This may be due to crowded seedbeds, the presence of raw organic matter high in carbon, or soil unfavorable for microbiological activity. The last-named condition may be expected particularly in nursery soils which have been disinfected with toxic substances. In such cases, the content of available nitrogen should be increased by the application of ammonia or nitrate fertilizers.

There is at hand no simple procedure for the determination of the exact amount of mineral nitrogen fertilizer to apply, since this depends upon a great variety of factors. Fortunately, deficiency of nitrogen is readily manifested by discoloration of foliage, and consequently the application of nitrogen fertilizers is usually dictated by the appearance of the stock. A good rule to follow in nitrogen fertilization is to apply too little rather than too much of the salt. A deficiency may be corrected by a second application. An excess results in planting stock of reduced vigor.

Table 5 gives the approximate range of annual applications of nitrogen salts in the form of ammonium sulphate which is likely to be required by different tree species.

TABLE 5.—*Approximate range (pounds per acre) of annual applications of elemental nitrogen or of nitrogen salt in the form of ammonium sulphate likely to be required by different tree species*

Forms of nitrogen	Jack pine	Norway pine	White pine	Spruce
	Pounds per acre	*Pounds per acre*	*Pounds per acre*	*Pounds per acre*
Elemental	10–20	20–40	30–50	40–60
20 percent ammonium sulphate	50–100	100–200	150–200	200–300

The analyses of the productive forest soils show that the nitrogen-phosphoric acid-potash ratio under natural conditions is about 1-2-5 for all four coniferous species studied. Experience in the greenhouse, as well as in the nursery, indicated that the nitrogen content should not greatly exceed the ratio of 2-2-5, if vigorous, well-balanced planting stock is to be produced. This means that in a soil with a phosphoric acid content of 40 pounds and a potash content of 100 pounds per acre, the allowable maximum of available nitrogen is 40 pounds per acre. Further studies are under way to confirm the extent of applicability of this ratio under a wider range of conditions.

The deficiency of phosphate, potash, and replaceable bases is corrected either through the application of organic remains or mineral

[4] WILDE, S. A. ADJUSTMENT OF SOIL FERTILITY IN CONIFEROUS NURSERIES. Wis. State Conserv. Dept. in Coop. with Wis. Agr. Col. Tech. Notes 19, 9 pp. 1937. [Mimeographed.]

fertilizers. The calculation of the necessary amount of fertilizing material to be applied is a matter of simple arithmetic. If, for example, a soil analyzes 30 pounds per acre of available phosphorus pentoxide and the desired content is 80 pounds, the deficiency of 50 pounds may be corrected by an addition of about 100 pounds of 45-percent treble superphosphate or 250 pounds of 20-percent superphosphate.

The suitability of different fertilizers for nursery use, the technique of their application, and the nutrient content of a variety of natural organic remains have been previously reported[5] (*14, 15, 16*).

DISCUSSION

Although the relation of the chemical composition of soil to tree growth has received considerable attention during the past 50 years, very little reliable information has been obtained. In most of the earlier experiments a number of important conditions were overlooked or misinterpreted. The extracting solutions used in chemical analysis were usually too strong, and hence the available nutrients were not determined. No regard was paid to the distribution and availability of nutrients in different soil horizons. The reactions between the nutrient salts, colloids, and other soil constituents were not considered. The studies dealt largely with a specific constituent and disregarded the influence of numerous other physical, chemical, and biological factors. The production of dry matter alone was studied, but the physiological and anatomical development of the seedlings was ignored. Particular attention was given to the production of luxuriant vegetative growth, but not necessarily to the development of vigorous seedlings resistant to diseases and unfavorable conditions of environment. As a result of this, until recently there has been little agreement among the leaders in silviculture on the problems of nursery-soil fertility and the use of fertilizers in general, or even on the role of single factors in seedling nutrition. Even in Germany, the country with the oldest silvicultural practice, the importance of nursery-soil fertilization has been fully recognized only during the past few years. The following translation of a few sentences from Dengler's modern and generally accepted text on silviculture (*5, pp. 420–421*), shows the confusion that has existed in German nursery practice.

> The soils of forest nurseries, permanent nurseries in particular, are impoverished in mineral nutrients by forest seedlings and require regular application of fertilizers, as do agricultural soils. At least as much fertilizing material must be returned to the nursery soil as has been taken from the soil by the young plants. The earlier, often expressed idea that the planting stock for poorer sites should not be encouraged by fertilization, which has gone so far that the commercial nurseries were offering stunted seedlings as being especially well suited for reforestation of the poorer soils, is positively and absolutely false.

The opposition to the use of fertilizers in forest nurseries was largely due to the fact that the results of fertilization were often confused with the results of unskilled fertilization, particularly fertilization with unbalanced or excessive nitrogen. Thus, past experience provides but little information on the problem of metabolism and

[5] WILDE, S. A. COMMERCIAL FERTILIZERS, THE USE OF LIQUID FERTILIZERS, AND PREPARATION AND USE OF COMPOSTED FERTILIZERS IN FOREST NURSERIES. Wis. State Conserv. Dept. in coop. with Wis. Agr. Col. Tech. Notes 7, 8, and 12, 25 pp., illus. 1936. [Mimeographed.]

correct use of fertilizers. The "new deal" in seedling nutrition is in its infancy, and it may be several years before the information presented in recent literature can be clarified and erroneous conclusions discarded. Particularly is this true in regard to greenhouse and sample plot studies carried on in this country and abroad during the past few years. For this reason, it is believed that a determination of nutrients and associated growth factors in productive forest soils provides more reliable general standards for the maintenance of fertility in nursery soils than are obtained by empirical trials.

SUMMARY

The soils under productive stands of representative coniferous species (*Pinus banksiana, P. resinosa, P. strobus,* and *Picea glauca*) were analyzed for pH value, exchange capacity, total and available nitrogen, available phosphorus, available potash, and replaceable bases. By means of statistical treatment of the data obtained, standards for the maintenance of fertility in coniferous nursery soils were established. The analyses of virgin soils showed that the nitrogen-phosphoric acid-potash ratio is about 1–2–5 for all four coniferous species studied. Typological investigations in Europe and greenhouse studies at the University of Wisconsin have indicated that the standards established for *Pinus resinosa* and *Picea glauca* may be safely applied to *Pinus sylvestris* and *Picea excelsa*, respectively.

General directions for the adjustment of soil conditions are outlined.

LITERATURE CITED

(1) Association of Official Agricultural Chemists.
1930. official and tentative methods of analysis . . . Compiled by the Committee on Editing Methods of Analysis. Ed. 3, 593 pp., illus. Washington, D. C.

(2) Bouyoucos, G. J.
1936. directions for making mechanical analyses of soils by the hydrometer method. Soil Sci. 42: 225–229, illus.

(3) Chapman, H. D., and Kelley, W. P.
1930. the determination of the replaceable bases and the base-exchange capacity of soils. Soil Sci. 30: 391–406.

(4) Clark, Noble, and Hoveland, Nieman, compilers.
1936. soils. Wis. Agr. Expt. Sta. Bull. 435 (Ann. Rept. 52): 88–99, illus.

(5) Dengler, Alfred.
1930. waldbau auf ökologischer grundlage, ein lehr- und handbuch. 560 pp., illus. Berlin.

(6) Harper, Horace J.
1924. the accurate determination of nitrates in soils. phenoldisulfonic method. Indus. and Engin. Chem. 16: 180–183.

(7) ———
1924. the determination of ammonia in soils. Soil Sci. 18: 409–418, illus.

(8) Němec, Antonín.
1937. der einfluss einseitiger stickstoffdüngung der waldbaumschulen auf die abgänge der verschulten fichtenpflanzen. Ceskoslov. Akad. Zeměděl. Sbornik (Czechoslovak, Acad. Agr. Ann.) 12: 461–469. [In Bohemian. German summary, pp. 468–469.]

(9) Russell, Sir E. John.
1932. soil conditions and plant growth. Ed. 6, 636 pp., illus. London, New York, [etc.].

(10) Snyder, E. F.
1928. methods for determining the hydrogen-ion concentration of soils. U. S. Dept. Agr. Cir. 56, 30 pp., illus. (Revised, 1935.)

(11) TRUOG, EMIL.
1930. THE DETERMINATION OF THE READILY AVAILABLE PHOSPHORUS OF SOILS. Jour. Amer. Soc. Agron. 22: 874–882.
(12) VOLK, N. J., and TRUOG, E.
1934. A RAPID CHEMICAL METHOD FOR DETERMINING THE READILY AVAILABLE POTASH OF SOILS. Jour. Amer. Soc. Agron. 26: 537–546.
(13) WILDE, S. A.
1935. THE SIGNIFICANCE OF SOIL TEXTURE IN FORESTRY AND ITS DETERMINATION BY A RAPID FIELD METHOD. Jour. Forestry 33: 503–508, illus.
(14) ———
1937. THE USE OF LIQUID HUMATE FERTILIZERS IN FOREST NURSERIES. Jour. Forestry 35: 388–392. illus.
(15) ——— BURAN, S. F., and GALLOWAY, H. M.
1937. NUTRIENT CONTENT AND BASE EXCHANGE PROPERTIES OF ORGANIC LAYERS OF FOREST SOILS IN THE LAKE STATES REGION. Soil Sci. 44: 231–239, illus.
(16) ——— and HULL, H. H.
1937. USE AND FUNCTIONS OF PEAT IN FOREST NURSERIES. Jour. Amer. Soc. Agron. 29: 299–313, illus.

U. S. GOVERNMENT PRINTING OFFICE: 1939

INDEX

Page

Acid preservatives, effect on cyanogenetic plants, analysis 87–88, 104
Acids, organic, casein in salts of, solubility and fractionation by means of these acids. Ralph H. McKee and Stephen P. Gould 125–143
Agaricaceae, wood-destroying, reactions on gallic and tannic acid media 689
Agrostis spp., infection by *Mastigosporium calvum* comb. nov., symptoms 291
Air, evaporating power, effect on growth of apples 112–113, 116–123
Alcohol—
ethyl, formation in growth of *Rhizopus* 893–894, 896
in dilution, effect on cyanogenetic plants, analysis 90–93, 104–105
Alfalfa—
coumarin-treated, feeding to rabbits, experiments 149, 151, 152–153
hay. *See* Hay, alfalfa; Hays, alfalfa.
root rot fungus, relation to stagonospora leaf spot disease, determinations 791–810
spoiled and treated, feeding to rabbits, experiments 150–153
stagonospora leaf spot and root rot, cultural studies 791–810
Alkalies, effect on cyanogenetic plants, analysis 88–89, 104
ALLARD, H. A.: Complete or Partial Inhibition of Flowering in Certain Plants When Days Are Too Short or Too Long 775–789
Alopecurus pratensis, infection by *Mastigosporium album* in Europe, symptoms 292
Amino—
acids, free, in discoloring cooked potatoes, study 435, 436, 439–440
nitrogen, in discoloring cooked potatoes, study 435, 436, 439–440
Ammonium—
bifluoride, feeding to grasshoppers, results 426, 427–428, 429
fluoride, feeding to grasshoppers, results 426, 427–428
Amphorophora rubi Kalt, mosaic vector, resistance in red raspberry to. Glenn A. Huber and C. D. Schwartze 623–633
ANDREWS, JOHN S.: Effect of Infestation With the Nematode *Cooperia curticei* on the Nutrition of Lambs 349–361
Aphelenchoides fragariae—
cause of strawberry dwarf 73–74
occurrence on strawberry plants in Massachusetts and North Carolina, study 74–80
two distinct strains occurring on strawberry plants in United States. J. R. Christie 73–80
Aphid resistance of red raspberry varieties, experiments 624–632
Aphids—
transmission of potato viruses, experiments 319–332
See also under hosts.
Apple fruit tissue, histology in relation to cracking. Leif Verner 813–824
Apples—
cracking and noncracking, difference in peripheral tissues 816–817
growth rate, apparatus for measuring, construction and use 110–112
Stayman Winesap—
densely shaded fruits, tissue modification 818–819
susceptibility to cracking, discussion 819–822
sunburned, spray-injured, and russeted, tissue modification 817–818
Winesap, enlargement rate and growth periodicity in relation to atmospheric conditions. C. P. Harley and M. P. Masure 109–124

Page

Armyworm, southern—
biological disposition of rotenone after ingestion. P. A. Woke 707–712
sixth-instar—
blood analysis. Frank H. Babers 697–706
hemolymph, chemical analysis 697–703
Arrowgrass, preservation for chemical analysis, studies 83–105
Arsenic—
entrance into wireworm body, discussion of method 236–237
trioxide, feeding to grasshoppers, results 426, 427–429
Arsenicals, reactions of wireworms to. Charles E. Woodworth 229–238
Ascomycetes, wood-destroying, reactions on gallic and tannic media 689
Ascorbic acid content of crown galls and contiguous tissue of tomato, determination 548
Aspergillus niger—
growth and reproduction, essentiality of gallium to. Robert A. Steinberg 569–574
trace-element requirements for growth and reproduction, deficiency tests 570–573
Atmosphere, conditions, relation to enlargement rate and periodicity of Winesap apples. C. P. Harley and M. P. Masure 109–124
Avena—
byzantina varieties, germination, tests 59–60, 62, 64, 65, 66
fatua—
feeding to livestock, experiments 477–481, 483, 501, 503–506, 511
varieties, germination tests 59–60, 62, 63, 66
germination, relation to kernel characters 66–68
growth habit, cold resistance, and maturity, relation to germination 68–69
harvested freshly, germination variability. F. A. Coffman and T. R. Stanton 57–72
kernel—
characters, relation to germination 66–68
size, relation to germination 68
kernels, primary and secondary, germination 69
moisture content, relation to germination 69–71
sativa, varieties, germination tests 59–66
species and varieties, germination tests 59–66
See also Oats.
Azotobacter—
chroöcoccum, growth—
and nitrogen fixation in liquid media 222–226
and nitrogen fixation on agar 219–222
effect of trace elements, cultural studies 462, 469–475
growth—
measurements 463
requirements 462
nitrogen-fixing ability, apparent induced loss. Charles R. Stumbo and P. L. Gainey 217–227
trace-element requirements, applicability of nutrient-solution purification to study of. Robert A. Steinberg 461–476

BABERS, FRANK H.: An Analysis of the Blood of the Sixth-Instar Southern Armyworm (*Prodenia eridania*) 697–706
Bacteria, decomposition of cyanogenetic plants, effect on hydrocyanic acid content 82
Baits—
arsenical, reaction of wireworms to, experiments 229–237
grasshopper, toxic and repellent properties of some inorganic and organic compounds and mixtures in. Charles H. Richardson and Edwin J. Seiferle 423–432

Page

Barley—
feeding to livestock, experiments 483, 493, 501, 511
hood and supernumerary spike development. O. T. Bonnett 371–377
natural selection, studies 189–199
varieties, mixture, natural selection in, effect. H. V. Harlan and M. L. Martini 189–199
BARRONS, KEITH C.: A Method of Determining Root Knot Resistance in Beans and Cowpeas in the Seedling Stage 363–370
BARTEL, A. T., and MARTIN, J. H.: The Growth Curve of Sorghum 843–849
Basswood, moisture movement in wood above fiber-saturation point, experiments 161–185
Bean, kidney, wild, flowering inhibition, effect of length of day, experiments 775–776, 779–788, 784–786, 787, 788
Beans—
classification on basis of root knot resistance 366–368
in seedling stage, resistance to root knot, determination method. Keith C. Barrons 363–370
lima, classification on basis of root knot resistance 366–368
root knot resistance—
field observations, agreement with seedling tests 369
in seedling stage, determination, materials and methods 363–364
Beef muscles, roasted, exterior temperature, effect upon press fluid, shear force, and cooking losses. Alice M. Child and Mary J. Satorius 865–871
Beetles. *See* under specific kinds.
BERRY, P. A.: Laboratory Studies on *Tetrastichus xanthomelaenae* Rond, and *Tetrastichus* sp., Two Hymenopterous Egg Parasites of the Elm Leaf Beetle 859–863
Beta-indole-acetic acid—
effect on development of crown gall 35–36
injection into tomato stems, experiments 34–35
Black ring, virus disease of cabbage and other crucifers. C. M. Tompkins, M. W. Gardner, and H. Rex Thomas 929–943
Blackfly, *Simulium nigroparvum*, digestive tract, morphology. James A. Cox 443–448
Blacks Mountain experimental forest, timber surveys, sampling error 715–735
BLAISDELL, DOROTHY J., DAVIDSON, ROSS W., and CAMPBELL, A. A.: Differentiation of Wood-Decaying Fungi by Their Reactions on Gallic or Tannic Acid Medium 683–695
Blister rust, damage, probable, in some representative stands of young western white pine, studies. Thomas W. Childs and J. W. Kimmey 557–568
Blue stem disease, effect on potato tubers, microchemical studies. L. M. Hill and C. R. Orton 387–392
Boneset, flowering, inhibition, effect of length of day, experiments 775–776, 780, 786–787, 788
BONNETT, O. T.: Hood and Supernumerary Spike Development in Barley 371–377
Boric acid, titration 903–907
Boron—
determination, methods of analysis 901–910
in natural phosphates, and defluorinated phosphate rocks, determination and occurrence. Lewis F. Rader, Jr., and W. L. Hill 901–916
Bran-water baits, toxic and repellent to grasshoppers, tests 423–432
Brassicae, black ring, studies 929–942
BRIESE, REINHOLD R., and COUCH, JAMES F.: Preservation of Cyanogenetic Plants for Chemical Analysis 81–107
BRINK, R. A., and SMITH, WILLIAM K.: Relation of Bitterness to the Toxic Principle in Sweetclover 145–154
British Columbia, white pine blister rust, damage to young trees, studies 558–568
Broomcorn, white Italian, preservation for chemical analysis, experiments 83–85, 88–92
BROWN, J. G., and BUTLER, KARL D.: Inflorescence Blight of the Date Palm 313–318

Page

Brown-staining fungi associated with engraver beetles (*Scolytus*) in white fir, further investigations. Ernest Wright 759–773
BRUNSON, M. H.: Influence of Japanese Beetle Instar on the Sex and Population of the Parasite *Tiphia popilliavora* 379–386
Bryophyllum pinnatum, inoculation for detection of growth substance in development of crown gall, experiments 23, 27–32, 34
BUCKMAN, STANLEY J., and REES, LOUIS W.: Moisture Movement in Wood Above the Fiber-Saturation Point 161–187
BUTLER, KARL D., and BROWN, J. G.: Inflorescence Blight of the Date Palm 313–318
Butterfat—
carotene content, relation to color and carotene content of roughage in dairy ration. R. E. Hodgson, J. C. Knott, H. K. Murer, and R. R. Graves 513–528
color, relation to color and carotene content of roughage in dairy ration. R. E. Hodgson, J. C. Knott, H. K. Murer, and R. R. Graves 513–528
vitamin A activity, relation to color and carotene content of roughage in dairy ration. R. E. Hodgson, J. C. Knott, H. K. Murer, and R. R. Graves 513–528
BYERS, HORACE G., GILE, PHILIP L., and LAKIN, HUBERT W., Effect of Different Soil Colloids and Whole Soils on the Toxicity of Sodium Selenate to Millet 1–20

Cabbage—
aphid, transmission of turnip mosaic virus 593–594
black ring—
disease, host range, list 934–939
disease, symptoms 930–933
of, a virus disease. C. M. Tompkins, M. W. Gardner, and H. Rex Thomas 929–943
virus, comparison with other crucifer viruses 941–942
virus, properties, studies 940
virus, technical description 940
virus, transmission 933–934
Chinese, mosaic virus, comparison with cabbage black ring virus 941–942
Calamagrostis spp., infection by *Mastigosporium calyum* comb. nov., symptoms 291
Calcium neutral salts, effect on carbon and nitrogen of soils. V. P. Sokoloff 201–216
California—
Blacks Mountain Experimental Forest, timber surveys, sampling error 715–735
ponderosa pine, rate of spread of surface fires in. John R. Curry and Wallace L. Fons 239–267
CAMPBELL, W. A., DAVIDSON, ROSS W., and BLAISDELL, DOROTHY J.: Differentiation of Wood-Decaying Fungi by Their Reactions on Gallic or Tannic Acid Medium 683–695
Cankers, blister rust, damage to young pine trees 561–568
Carbohydrate content of roots of bearing pecan trees, seasonal variations. C. L. Smith and J. G. Waugh 449–460
Carbohydrates, lactic acid production from, by *Rhizopus* pellicles 891, 895–896
Carbon—
content of soils, effect of neutral salts of sodium and calcium. V. P. Sokoloff 201–216
relationships in developmental stages of *Rhizopus*, studies 878–879
Carotene—
content of—
butterfat, determination 514, 522, 524–525
butterfat, relation to color and carotene content of roughage in dairy ration. R. E. Hodgson, J. C. Knott, H. K. Murer, and R. R. Graves 513–528
cattle feeds, determination, analytical procedure 638–648
cattle feeds, determination, comparison of different methods 648–652
corn silage, determination 661–666
dairy feeds, determination 514–522, 524–525

Carotene—Continued. Page
content of—Continued.
market hays and corn silage. Herbert G. Wiseman, Edward A. Kane, Leo A. Shinn, and C. A. Cary 635–669
determinations in cattle feeding, practical significance 659–661
CARY, C. A., WISEMAN, HERBERT G., KANE, EDWARD A., and SHINN, LEO A.: The Carotene Content of Market Hays and Corn Silage 635–669
Casein—
acid-precipitated—
fractionation investigations 131–139, 140–141
solubility, experiments 126–131
fractionation—
and solubility in salts of certain organic acids. Ralph H. McKee and Stephen P. Gould 125–143
with sodium benzenesulphonate solutions 133–137, 140–141
with sodium cymenesulphonate solution, investigations 138, 139, 140–141
solubility in—
potassium thiocyanate solutions, determination 130, 140
salts of certain organic acids, and its fractionation by means of these acids. Ralph H. McKee and Stephen P. Gould 125–143
sodium benzenesulphonate solutions, determination 131, 139–141
solutions of recrystallized sodium cymenesulphonate, determination 129–130, 139–141
Caseins, definition 125
Catalase activity in crown galls and contiguous tissue of tomato, sugar beet, and red raspberry 548–549, 552, 553
Cattle—
feeding—
carotene determinations in, practical significance 659–661
experiments 477–512
sweetclover disease, description 145
Cauliflower mosaic virus, comparison with cabbage black ring virus 941–942
Cecil sandy clay loam, effect on toxicity of sodium selenate to millet 7–14
CHAMBERS, EVELYN, WOODRUFF, SYBIL, and KLAAS, HELEN: A Study of Protein Extract From Soybeans With Reference to Its Use in Food 737–746
Chemical elements, biological essentiality and atomic structure, correlations between. Robert A. Steinberg 851–858
Cherry, wild, preservation for chemical analysis, studies 83–105
Chicks—
energy transformations at various temperatures, efficiency 541–542
fasting katabolism, study 539–542
food—
calorigenic action at various environmental temperatures 540–541
consumption, excretion, and availability of nutrients, effect of temperature 534–536
gains in body substance, composition and energy content, determination 539
growth rate, effect of temperature, experiments 531–533
mortality, effect of temperature, experiments 531
nitrogen, carbon, and energy balance, determination 537–539
respiratory exchange, effect of temperature 536–537
White Leghorn—
growth rate, effect of environmental temperature on. C. F. Winchester and M. Kleiber 529–544
mortality, effect of environmental temperature on. C. F. Winchester and M. Kleiber 529–544
utilization of food energy, effect of environmental temperature on. C. F. Winchester and M. Kleiber 529–544

Page
CHILD, ALICE M., and SATORIUS, MARY J.: Effect of Exterior Temperature Upon Press Fluid, Shear Force, and Cooking Losses of Roasted Beef and Pork Muscles 865–871
CHILDS, THOMAS W., and KIMMEY, J. W.: Studies on Probable Damage by Blister Rust in Some Representative Stands of Young Western White Pine 557–568
Chloroform mixtures, effect on cyanogenetic plants, analysis 87
Chlorosis, of gardenia, relation to soil temperature. Linus H. Jones 611–621
CHRISTENSEN, F. W., and HOPPER, T. H.: Digestible Nutrients and Metabolizable Energy in Certain Silages, Hays, and Mixed Rations 477–512
CHRISTIE, J. R.: Two Distinct Strains of the Nematode *Aphelenchoides fragariae* Occurring on Strawberry Plants in the United States 73–80
Clover—
red, plants—
moderately resistant to powdery mildew infection, study 680–681
resistant and susceptible to powdery mildew, *Erysiphe polygoni*, host-parasite relations. Oliver F. Smith 671–682
resistant to powdery mildew infection, study 677–680
susceptible to powdery mildew infection, study 675–677
Clovers, stagonospora leaf spot and root rot, cultural studies 791–810
Clutches, in Rhode Island Red pullets, time interval between. F. A. Hays 575–581
COFFMAN, F. A., and STANTON, T. R.: Variability in Germination of Freshly Harvested *Avena* 57–72
Colloids, soil, effect on toxicity of sodium selenate to millet. Philip L. Gile, Hubert W. Lakin, and Horace G. Byers 1–20
Color—
in gardenia leaves, effects of soil temperatures, experiments 611–621
of butterfat, relation to color content of roughage in dairy ration. R. E. Hodgson, J. C. Knott, H. K. Murer, and R. R. Graves 513–528
Columbiana soils, effects on toxicity of sodium selenate to millet 4, 6, 7, 9, 12, 16, 17–18
Combustion of wood, theoretical aspects, discussion 249–250
Compounds, inorganic and organic, toxic and repellent properties in grasshopper baits. Charles H. Richardson and Edwin J. Seiferle 423–432
Concentrates, feeding to livestock, experiments 478, 483, 493, 501, 502–511
Conifers—
northern, growing in forest nurseries, soil-fertility standards. S. A. Wilde 945–952
See also Pine; *Pinus;* and under specific kinds.
Cooking roasts, beef and pork, effect of exterior temperature on 865–871
Cooperia curticei, nematode, infestation of lambs, effect on nutrition. John S. Andrews 349–361
Corn—
damage by the muskrat in central Iowa, observations. Paul L. Errington 415–421
silage—
carotene content. Herbert G. Wiseman, Edward A. Kane, Leo A. Shinn, and C. A. Cary 635–669
carotene content, determination 661–666
feeding to livestock, experiments 477–483, 486, 490–491, 497, 501–508
Cotton—
American wild, leaf and stem, anatomical studies 269–283
fiber—
improvement studies in Gila Valley, Ariz 583–587
length and maturity, relation between. R. S. Hawkins 583–587
length, calculations 45–46, 53–55

Cotton—Continued. Page
lint—
fiber lengths, measurement methods in present use 41-43
sorting by photoelectric apparatus, experiments 46-53
New World, leaf and stem, anatomical studies 269-283
Old World, leaf and stem, anatomical studies 269-283
plants, twin—
cytology. J. M. Webber 155-160
types, description 155-158
polyembryony, cytological study 155-159
seed, fibers, length measurements, photoelectric sorter for. Burt Johnson 41-56
species and related genera, leaf and stem, anatomical studies 269-283
See also *Gossypium*.
Cottonseeds, polyembryony, occurrence and study 155-159
COUCH, JAMES F., and BRIESE, REINHOLD R.: Preservation of Cyanogenetic Plants for Chemical Analysis 81-107
Coumaric acid, feeding to rabbits, experiments 150, 152
Coumarin, feeding to rabbits, experiments 151-153
Cowpeas—
classification on basis of root knot resistance 366-368
in seedling stage, resistance to root knot, determination method. Keith C. Barrows 363-370
root knot resistance—
field observations, agreement with seedling tests 369
in seedling stage, determination, materials, and methods 363-364
Cows, dairy, carotene consumption 518-522, 524-525
COX, JAMES A.: Morphology of the Digestive Tract of the Blackfly (*Simulium nigroparvum*) 443-448
Cress, hoary. See *Lepidium draba*.
Cronartium ribicola, infection of white pine, intensity, relation to degree of damage 557-568
Crops—
damage by muskrat in central Iowa, observations. Paul L. Errington 415-421
green silage, chemical composition 483-487
Crown gall—
and contiguous tissue, physiological studies. R. Nagy, A. J. Riker, and W. H. Peterson 545-555
bacteria, production of growth substance in culture, study 33
development, effect of beta-indole-acetic acid 35-36
growth substance, and development. S. B. Locke, A. J. Riker, and B. M. Duggar 21-39
See also under specific host.
Crucifer—
mosaic viruses, comparison with turnip mosaic virus from New York 598-599
viruses, comparison with cabbage black ring virus 941-942
Cruciferae, susceptibility to turnip mosaic infection, experiments 598-599
Crucifers, black ring, a virus disease. C. M. Tompkins, M. W. Gardner, and H. Rex Tompkins 929-943
CURRY, JOHN R., and FONS, WALLACE L.: Rate of Spread of Surface Fires in the Ponderosa Type of California 239-267
Cyanogenesis, factors involved, study 81-82
Cyanogenetic plants, preservation for chemical analysis. Reinhold R. Briese and James F. Couch 81-107
Cytology of twin cotton plants. J. M. Webber 155-160

Dactylis glomerata, infection by *Mastigosporium calvum* comb. nov., symptoms 290-291
Dairy ration, roughage in, color and carotene content, relation to color, carotene content, and vitamin A activity of butterfat. R. E. Hodgson, J. C. Knott, H. K. Murer, and R. R. Graves 513-528

Date palm— Page
inflorescence blight—
J. G. Brown and Karl D. Butler 313-318
cause 314-315
conditions affecting 316-317
symptoms 314
Khamedj, symptoms 317
See also *Phoenix dactylifera*.
DAVIDSON, ROSS W., CAMPBELL, W. A., and BLAISDELL, DOROTHY J.: Differentiation of Wood-Decaying Fungi by Their Reactions on Gallic or Tannic Acid Medium 683-695
Day length, effect on flowering in certain plants 775-789
Deschampsia caespitosa, infection by *Mastigosporium album* in Europe, symptoms 292
Digestive tract, of blackfly, *Simulium nigroparvum*, morphology. James A. Cox 443-448
Dilophospora alopecuri cultures from *Holcus lanatus*, study 293
DUGGAR, B. M., LOCKE, S. B., and RIKER, A. J.: Growth Substance and the Development of Crown Gall 21-39
DYKSTRA, T. P., and WHITTAKER, W. C.: Experiments on the Transmission of Potato Viruses by Vectors 319-334

Egg production in Rhode Island Red pullets, relation to time interval between clutches, studies 575-581
Elements, chemical, biological essentiality and atomic structure, correlations between. Robert A. Steinberg 851-858
Elm leaf beetle, two hymenopterous egg parasites, *Tetrastichus xanthomelaenae* Rond and *Tetrastichus* sp., laboratory studies. P. A. Berry 859-863
Energy—
metabolism of—
chicks, effect of temperature 537-539
lambs infested with *Cooperia curticei* 357-358, 359, 360
metabolizable—
in certain silages, hays, and mixed rations. F. W. Christensen and T. H. Hopper 477-512
in feeds and rations, determination 507-511
of chicks, efficiency of transformation at various temperatures 541-542
Engraver beetles, in white fir, association with brown-staining fungi, further investigations. Ernest Wright 759-773
ERRINGTON, PAUL L.: Observations on Muskrat Damage to Corn and Other Crops in Central Iowa 415-421
Erysiphe polygoni—
infection of susceptible red clover plants 675-677
powdery mildew, host-parasite relations in resistant and susceptible red clover plants 671-682
spore germination and early stages of infection of red clover 673-675
Eupatorium torreyanum, flowering inhibition, effect of length of day, experiments 775-776, 780, 786-787, 788

Feed—
constituents in mixed rations, digestibility coefficients 500-507
livestock, chemical composition 483-487
Feeds—
cattle, carotene content—
determination, analytical procedure 638-648
determination, comparison of different methods 648-652
experiments 635-667
dairy cattle, color and carotene content, relation to color, carotene content, and vitamin A activity of butterfat 513-526
livestock—
digestible nutrients and metabolizable energy 477-512
metabolizable energy, determination 507-511
See also Rations; and under specific kinds.
Fertility, soil, standards for growing northern conifers in forest nurseries. S. A. Wilde. 945-952

Page

Fertilizer treatment, of potato, relation to foliar diagnosis. Walter Thomas and Warren B. Mack 397–414
Feterita, Spur, preservation for chemical analysis, experiments 83–87, 90–92, 95, 103
Filtrate factor in certain milk products, assays. T. H. Jukes and G. A. Richardson 603–610
Fir, white, brown-staining fungi associated with engraver beetles (*Scolytus*) in, further investigations. Ernest Wright 759–773
Fires, surface, rate of spread in ponderosa pine type of California. John R. Curry and Wallace L. Fons 239–267
Flowering, in certain plants, complete or partial inhibition, when days are too short or too long. H. A. Allard 775–789
Foliar diagnosis—
definition 397
of potato, in relation to development and fertilizer treatment. Walter Thomas and Warren B. Mack 397–414
FONS, WALLACE L., and CURRY, JOHN R.: Rate of Spread of Surface Fires in the Ponderosa Pine Type of California 239–267
Forage—
legumes, root rot of, relation to stagonospora leaf spot. Fred Reuel Jones and J. L. Weimer 971–812
plants, cyanogenetic, preservation for chemical analysis, experiments 81–105
Forest—
fires, rate of spread in ponderosa pine type of California, study 239–266
nurseries—
growing northern conifers in, soil-fertility standards. S. A. Wilde 945–952
soil conditions, adjustments, general directions 948–951
soil improvement principles 948
FOSTER, J. W., and WAKSMAN, SELMAN A.: Respiration and Lactic Acid Production by a Fungus of the Genus *Rhizopus* 873–899
Fungi—
brown-staining associated with engraver beetles (*Scolytus*) in white fir, further investigations. Ernest Wright 759–773
wood-decaying, differentiation by their reactions on gallic or tannic acid medium. Ross W. Davidson, W. A. Campbell, and Dorothy J. Blaisdell 683–695
Fungus of genus *Rhizopus*, respiration and lactic acid production. Selman A. Waksman and J. W. Foster 873–899
Fusarium—
moniliforme, inoculation of *Phoenix dactylifera* with, studies 315–316, 317–318
spp., cause of inflorescence blight of date palms 314–316, 317

GAINEY, P. L., and STUMBO, CHARLES R.: An Apparent Induced Loss of Nitrogen-Fixing Ability in *Azotobacter* 217–227
Gallic acid medium, reactions of wood-decaying fungi on, differentiation. Ross W. Davidson, W. A. Campbell, and Dorothy J. Blaisdell 683–695
Gallium, essentiality to growth and reproduction of *Aspergillus niger*. Robert A. Steinberg 569–574
Galls, crown, growth substances from, effect upon tomato tissues inoculated with attenuated strain 34
Gardenia—
chlorosis, relation to soil temperature. Linus H. Jones 611–621
leaf, chlorotic, effect of soil temperature on size, experiments 615–616
leaves, color, effects of soil temperatures, experiments 611–621
plant, effect of soil temperature, experiments 614
plants, chlorotic, chemical analysis of leaves and twigs 614–615
GARDNER, M. W., TOMPKINS, C. M., and THOMAS, H. REX.: Black Ring, a Virus Disease of Cabbage and Other Crucifers 929–943

Page

Germination, variability in *Avena* when freshly harvested. F. A. Coffman and T. R. Stanton 57–72
GILE, PHILIP L., LAKIN, HUBERT W., and BYERS, HORACE G.: Effect of Different Soil Colloids and Whole Soils on the Toxicity of Sodium Selenate to Millet 1–20
Glutathione content of crown galls and contiguous tissue of tomato, determination 548
Gossypium—
barbadense, haploid-diploid twins, cytology 157–159
hirsutum, diploid twins, cytology 156, 159
leaf—
anatomy 270–277
and stem, anatomy. Irma E. Webber 269–286
epidermis, description 270–273
hypodermis, description 273–274
mesophyll, description 274–275
nectaries, occurrence and description 275–276
petioles, description 276–277
venation, description 275
nanking × *G. thurberi*, diploid twins, cytology 156, 158–159
stem—
anatomy 278–283
cortex and pericycle, description 278–279
epidermis and periderm, description 278
phloem and xylem, description 279–280
pith, description 280
shape and tissue arrangement 278
See also Cotton.
GOULD, STEPHEN P., and MCKEE, RALPH H.: The Solubility of Casein in Salts of Certain Organic Acids, and Its Fractionation by Means of These Acids 125–143
Gramineae, two mastigosporium leaf spots on. Roderick Sprague 287–299
Grasses—
inoculation with *Mastigosporium album* and *M. calvum* comb. nov., studies 293–294
two mastigosporium leaf spots on, experiments 287–299
Grasshopper baits, toxic and repellent properties of some inorganic and organic compounds and mixtures in. Charles H. Richardson and Edwin J. Seiferle 423–432
Grasshoppers—
feeding compounds and mixtures in bran baits, results 425–428
mortality, bait consumption, and feeding time, theoretical combinations 425–431
GRAVES, R. R., HODGSON, R. E., KNOTT, J. C., AND MURER, H. K.: The Relation of Color and Carotene Content of Roughage in the Dairy Ration to the Color, Carotene Content, and Vitamin A Activity of Butterfat 513–528
Ground slope, effect on rate of spread of fires in ponderosa pine stands, tests 257–260, 266

Hackberry wood, moisture movement above fiber-saturation point, experiments 161–185
HARLAN, H. V., and MARTINI, M. L.: The Effect of Natural Selection in a Mixture of Barley Varieties 189–199
HARLEY, C. P., and MASURE, M. P: Relation of Atmospheric Conditions to Enlargement Rate and Periodicity of Winesap Apples 109–124
HASEL, A. A.: Sampling Error in Timber Surveys 713–736
HAWKINS, R. S.: Relation Between Fiber Length and Maturity in Cotton 583–587
Hay—
alfalfa—
coumarin treated, feeding to rabbits, experiments 149, 151, 152–153
feeding to livestock, experiments 480, 482, 485, 491–493, 501–511
leafy portion, vitamin D content, comparison with content of stemmy portion 393–395
spoiled and treated, feeding to rabbits, experiments 149–151, 152–153
stemmy and leafy portions, vitamin D potency, comparison. G. C. Wallis 393–396

Page

Hay—Continued.
in dairy ration, color and carotene content, relation to color, carotene content, and vitamin A activity of butterfat 513–526
Johnson grass, preservation for chemical analysis, experiments 83, 96–97, 98, 103
Sweetclover, feeding to livestock, experiments 477–481, 482, 491–493, 502
HAYS, F. A.: Time Interval Between Clutches in Rhode Island Red Pullets 575–581
Hays—
alfalfa, carotene content of various grades 652–658
clover, carotene content of two grades, determination 659
digestible nutrients and metabolizable energy. F. W. Christensen and T. H. Hopper 477–512
market, carotene content—
Herbert G. Wiseman, Edward A. Kane, Leo A. Shinn, and C. A. Cary 635–669
determination 650–659
sweetclover, feeding to rabbits, experiments 146–149, 152
timothy, carotene content of various grades, determination 658–659
Heartwood, moisture movement above fiber-saturation point, experiments 161–185
Hegari—
Dwarf, growth curve 843–849
preservation for chemical analysis, experiments 83–85, 88, 90–91, 93, 95, 101, 103
Hemicellulose content of roots of pecan tree, seasonal variation 456, 458
Hemolymph of sixth-instar armyworm, analysis 697–703
Hempweed, climbing, flowering, inhibition, effect of length of day, experiments 775–779, 782, 784, 787, 788
Hibiscus spp., leaf and stem anatomy, investigations 270–283
Hickory wood, moisture movement above fiber-saturation point, experiments 161–185
HILL, L. M., and ORTON, C. R.: Microchemical Studies of Potato Tubers Affected With Blue Stem Disease 387–392
HILL, W. L., and RADER, LEWIS F., JR.: Determination and Occurrence of Boron in Natural Phosphates, Superphosphates, and Defluorinated Phosphate Rocks 901–916
HODGSON, R. E., KNOTT, J. C., MURER, H. K., and GRAVES, R. R.: The Relation of Color and Carotene Content of Roughage in the Dairy Ration to the Color, Carotene Content, and Vitamin A Activity of Butterfat 513–528
Honeydew, volume excreted by pea aphid, factors affecting 836–838
HOPPER, T. H., and CHRISTENSEN, F. W.: Digestible Nutrients and Metabolizable Energy in Certain Silages, Hays, and Mixed Rations 477–512
Horse strongyle—
eggs—
burial in soils, procedure and apparatus 335–338
content of feces buried and percentage of development in cultures 338–339
depth of burial, effect on distribution and number of larvae 342–343
larvae—
distribution and number in feces and soil, factors affecting 342–343
infective, developing in feces buried in different soils, vertical migration, distribution, and survival. John T. Lucker 335–348
number recovered from soil, relation to number of eggs buried 343–344, 346–347
vertical migration, degree in various types of soil 339–341
Horses, parasitism by strongyles, study 335–347
Host-parasite relations in red clover plants resistant and susceptible to powdery mildew, *Erysiphe polygoni*. Oliver F. Smith 671–682
HUBER, GLENN A., and SCHWARTZE, C. D.: Resistance in the Red Raspberry to the Mosaic Vector *Amphorophora rubi* Kalt 623–633
Hydnaceae, wood-destroying, reactions on gallic and tannic acid media 686–687

Page

Hydrocyanic acid—
content of plant specimens, analysis, methods 84
formation and preservation in plants 81–82
recovery from mercuric cyanide, experiments 102–104
Hydrogen-ion concentration in crown galls and contiguous tissue of tomato, sugar beet, and red raspberry 551–552

Idaho, white pine blister rust, damage to young trees, studies 559–568
Inflorescence blight of date palm. J. G. Brown and Karl D. Butler 313–318
Inheritance, in sorghums, Q B Gs group, linkage studies 747–755
Insect vectors of potato viruses, testing 322–324, 325–328, 331–332
Insects, transmission of potato virus diseases, experiments 322–324, 325–328, 331–332
Iodoacetic acid, influence on glycolysis by *Rhizopus* 891–893, 895
Iowa, central, muskrat damage to corn and other crops in, observations. Paul L. Errington 415–421

Japanese beetle—
abundance and instars, effect on *Tiphia popilliavora* population 384, 385–386
instar—
influence on sex and population of parasite *Tiphia popilliavora*. M. H. Brunson 379–386
relation to sex of progeny of parasitizing *Tiphia popilliavora* 381–383, 385
larvae—
instar preferred for oviposition by *Tiphia popilliavora* 381
third-instar, number, fluctuations and effect on *Tiphia* population 384–386
life cycle, relation to *Tiphia* oviposition 380, 381, 385
parasitized by *Tiphia popilliavora*, life cycle 380
JODIDI, SAMUEL L.: Preliminary Biochemical Studies on Effects of Certain Environmental Factors on Development and Composition of the Peanut 301–311
JOHNSON, BURT: A Photoelectric Sorter for Length Measurements of Fibers of Seed Cotton 41–56
Johnson grass, dried, preservation for chemical analysis, studies 83, 96–97, 98, 103
JONES, FRED REUEL, and WEIMER, J. L.: Stagonospora Leaf Spot and Root Rot of Forage Legumes 791–812
JONES, LINUS H.: Relation of Soil Temperature to Chlorosis of Gardenia 611–621
JUKES, T. H., and RICHARDSON, G. A.: Assays of Riboflavin and the Filtrate Factor in Certain Milk Products 603–610

Kalanchoe diagremontiana, inoculation for detection of growth substance in development of crown gall, experiment 23, 25–31
KANE, EDWARD A., WISEMAN, HERBERT G., SHINN, LEO A., and CARY, C. A.: The Carotene Content of Market Hays and Corn Silage 635–669
Katabolism, fasting, of chicks, determination 530–542
Khamedj, infection of date palms, symptoms 317
KIMMEY, J. W., and CHILDS, THOMAS W.: Studies on Probable Damage by Blister Rust in Some Representative Stands of Young Western White Pine 557–568
KLAAS, HELEN, WOODRUFF, SYBIL, and CHAMBERS, EVELYN: A Study of Protein Extract From Soybeans With Reference to Its Use in Food 737–746
KLEIBER, M., and WINCHESTER, C. F.: The Effect of Environmental Temperature on Mortality, Rate of Growth, and Utilization of Food Energy in White Leghorn Chicks 529–544

Page

KNOTT, J. C., HODGSON, R. E., MURER, H. K., and GRAVES, R. R.: The Relation of Color and Carotene Content of Roughage in the Dairy Ration to the Color, Carotene Content, and Vitamin A Activity of Butterfat 513-528
Kokia spp.; leaf and stem anatomy, investigations 270-283

Lactic acid, production by a fungus of the genus *Rhizopus*. Selman A. Waksman and J. W. Foster 873-899
Lactoflavin, content of milk products, biological assay 606-608
LAKIN, HUBERT W., GILE, PHILIP L., and BYERS, HORACE G.: Effect of Different Soil Colloids and Whole Soils on the Toxicity of Sodium Selenate to Millet 1-20
Lambs—
infestation with *Cooperia curticei*, experiments, materials and methods 350-353
infested with *Cooperia curticei*—
energy metabolism 357-358, 359, 360
feed, apparent digestibility coefficients 355-356, 358
feed consumption and weights 355, 358
nitrogen and mineral metabolism 356-357
nutrition, effect of infestation with nematode *Cooperia curticei*. John S. Andrews 349-361
Leaf-spot, stagonospora, relation to root rot of forage legumes. Fred Reuel Jones and J. L. Weimer 791-812
Legumes, forage, root rot of, relation to stagonospora leaf spot. Fred Reuel Jones and J. L. Weimer 791-812
Lepidium draba—
adventitious shoot development and root formation 927
anatomical development. Austin O. Simonds 917-928
crown, anatomy 925-926
embryo, anatomy 919-920
leaf, anatomy 927-928
seedling—
external morphology 917-919
root-stem transition, anatomy 923-924
root system, anatomy 920-923
stem, anatomy 924-925
Leptosphaeria pratensis, ascigerous form of *Stagonospora meliloti* 808-809
Linseed meal, feeding to livestock, experiments 485, 493, 500-502
Livestock feeds, digestible nutrients and metabolizable energy 477-512
LOCKE, S. B., RIKER, A. J., and DUGGAR, B. M.: Growth Substance and the Development of Crown Gall 21-39
LUCKER, JOHN T.: Vertical Migration, Distribution, and Survival of Infective Horse Strongyle Larvae Developing in Feces Buried in Different Soils 335-348

MACK, WARREN B., and THOMAS, WALTER: Foliar Diagnosis in Relation to Development and Fertilizer Treatment of the Potato 397-414
MCKEE, RALPH H., and GOULD, STEPHEN P.: The Solubility of Casein in Salts of Certain Organic Acids and Its Fractionation by Means of These Acids 125-143
Macrosiphum solanifolii, transmission of potato viruses, experiments 319-323, 325-327, 328-332
Manure, rotted, of horse, treatment of potato plants, effects, foliar diagnosis 398-414
Maple wood, moisture movement above fiber-saturation point, experiments 161-185
Marshall silt loam, effect on toxicity of sodium selenate to millet 7-14
MARTIN, J. H., and BARTEL, A. T.: The Growth Curve of Sorghum 843-849
MARTINI, M. L., and HARLAN, H. V.: The Effect of Natural Selection in a Mixture of Barley Varieties 189-199
Massachusetts, Wareham, experiments with strawberry dwarf 74-77, 78-80

Page

Mastigosporium—
album—
symptoms caused by in Europe 291-292
taxonomy 295-298
var. *athrix*, relationship to *Septogloeum athrix* comb. nov 298
calvum comb. nov.—
distribution and ecology in western United States 287-290
infection of *Agrostis* spp., symptoms 291
infection of *Calamagrostis* spp., symptoms 291
infection of orchard grass, symptoms 290-291
morphology 294-295
pure-culture studies 292-293
synonymy 298
taxonomy 295-298
emended description 296
leaf spots, two on Gramineae. Roderick Sprague 287-299
spp., inoculation studies 293-294
MASURE, M. P., and HARLEY, C. P.: Relation of Atmospheric Conditions to Enlargement Rate and Periodicity of Winesap Apples 109-124
Meats, cooking, effect of exterior temperature upon 865-871
Medicago spp., stagonospora leaf spot and root rot fungi, cultural studies 791-810
Melilotic acid—
feeding to rabbits, experiments 150, 152
lactone, feeding to rabbits, experiments 150-151, 152
Melilotus—
dentata, spoiled, feeding to rabbits, experiments 147-148, 152-153
spp., stagonospora leaf spot and root rot fungi, cultural studies 791-810
Mercuric—
chloride, effect on hydrocyanic acid content of plants, experiments 93-101
cyanide, effect on hydrocyanic acid content of plants, experiments 102-104
Metabolism—
energy. *See* Energy.
lambs infested with *Cooperia curticei*, studies 356-358, 359, 360
mineral, of lambs infested with *Cooperia curticei* 356-357
nitrogen, of lambs infested with *Cooperia curticei* 356
trials with sheep and steers 479-480
See also under specific kinds.
Metals, heavy—
and rare, effect on growth and lactic acid production by *Rhizopus* 882-887, 896-897
See also Trace element.
Mikania scandens, flowering, inhibition, effect of length of day, experiments 775-779, 782-784, 787, 788
Mildew, powdery, *Erysiphe polygoni*, host-parasite relations in resistant and susceptible red clover plants. Oliver F. Smith 671-682
Milk products—
filtrate factor, biological assay 604-606
riboflavin and filtrate factor in assays. T. H. Jukes and G. A. Richardson 603-610
spray-dried, preparation 603-604
Millet—
plants, selenium content, experiments 14-17
toxicity of sodium selenate to, effect of different soil colloids and whole soils on. Philip L. Gile, Hubert W. Lakin, and Horace G. Byers 1-20
Milo, Double Dwarf Yellow, growth curve 843-849
Mixtures, inorganic and organic, toxic and repellent properties in grasshopper baits. Charles H. Richardson and Edwin J. Seiferle 423-432
Moisture—
effect on—
development of *Trichosporium symbioticum* and *Spicaria anomala*, tests 766-767
spread of fire in ponderosa pine stands, tests 241-242, 245, 248-249, 252-253, 254-256, 260-266

Moisture—Continued. Page
movement in wood above fiber-saturation point. Louis W. Rees and Stanley J. Buckman 161-187
Mosaic—
disease of turnip. C. M. Tompkins 589-602
of potato, transmission experiments 319-332
vector, *Amphorophora rubi* Kalt, resistance in red raspberry to. Glenn A. Huber and C. D. Schwartze 623-633
virus. *See* under host.
MURER, H. K., HODGSON, R. E., KNOTT, J. C., and GRAVES, R. R.: The Relation of Color and Carotene Content of Roughage in the Dairy Ration to the Color, Carotene Content, and Vitamin A Activity of Butterfat 513-528
Muskrat—
damage to corn—
and other crops in central Iowa, observations. Paul L. Errington 415-421
evaluation of instance 418-421
food habits, investigation 415-416, 421
Muskrats—
cornfield-feeding, seasonal changes in habits 416-418, 420-421
foraging tendencies 416, 420-421
Mustard plant, tolerance to sodium selenate in quartz sand 17
Myzus—
circumflexus, transmission of potato viruses, experiments 319-323, 325-327, 328-332
persicae, transmission of—
mosaic virus 593-594
potato viruses, experiments 319-323, 325-327, 328-332
solani, transmission of potato viruses, experiments 319-323, 325-327, 328-332

NAGY, R., RIKER, A. J., and PETERSON, W. H.: Some Physiological Studies of Crown Gall and Contiguous Tissue 545-555
Nematode, *Aphelenchoides fragariae*, two distinct strains occurring on strawberry plants in United States. J. R. Christie 73-80
Nitrogen—
amino, in discoloring potatoes, study 435, 436, 439-440
content of—
potato leaf, after treatment with complete and incomplete fertilizers, study 402-414
roots of bearing pecan trees, seasonal variations. C. L. Smith and J. G. Waugh 449-460
roots of pecan tree, seasonal variation 456-457, 459
soils, effect of neutral salts of sodium and calcium. V. P. Sokoloff 201-216
fixing ability in *Azotobacter*, apparent induced loss. Charles R. Stumbo and P. L. Gainey 217-227
North Carolina, Willard, experiments with strawberry dwarf 74-76, 77-80
Nurseries, forest. *See* Forest nurseries.
Nutrient-solution purification—
applicability to study of trace-element requirements of rhizobium and azotobacter. Robert A. Steinberg 461-476
effect on trace-element requirements of *Aspergillus niger*, tests 570-573
Nutrients, digestible, in certain silages, hays, and mixed rations. F. W. Christensen and T. H. Hopper 477-512
Nutrition—
effect on development of *Trichosporium symbioticum* and *Spicaria anomala*, tests 768-769
of lambs, effect of infestation with nematode *Cooperia curticei*. John S. Andrews 349-361

Oak wood, moisture movement above fiber-saturation point, experiments 161-185
Oats—
inoculation for detection of growth substance in development of crown gall, experiments 23, 33, 35-36
Oats—Continued. Page
wild—
feeding to livestock, experiments 477-481, 483, 501, 503-506, 511
varieties, germination tests 59-60, 62, 63, 66
See also *Avena*.
Orchard grass, infection by *Mastigosporium calvum* comb. nov., symptoms 290-291
Organic—
acids, casein in salts of, solubility and fractionation by means of these acids. Ralph H. McKee and Stephen H. Gould 125-143
matter in soils, conservation, effect of neutral salts, experiments 201-215
ORTON, C. R., and HILL, L. M.: Microchemical Studies of Potato Tubers Affected With Blue Stem Disease 387-392
Oven temperatures, effect on press fluid, shear force, and cooking losses during wasting of beef and pork muscle, determination 865-871
Oxidase activity in crown galls and contiguous tissue of tomato, sugar beet, and red raspberry 549, 552, 553
Oxygen, influence on lactic acid production by *Rhizopus* 887-890, 895-896

Palm, date. *See* Date palm.
Parasite-host relations in red clover plants resistant and susceptible to powdery mildew, *Erysiphe polygoni*. Oliver F. Smith 671-682
Pea aphid—
alate—
and apterous, quantitative chemical analyses 832-835
progeny, maximum production and conditions inducing it 828-830
food source, nature of food, and structure of digestive tract 835-836
honeydew excreted, volume, factors affecting 836-838
starving, effect on winged forms of progeny 831-832, 838-839
wing—
appearance, association with decreased volume of honeydew and concentration of body contents 837-838
development, physiological conditions producing. Carl W. Schaefer 825-841
Peach aphid, green, transmission of mosaic virus 593-594
Peanut—
composition and development, effects of certain environmental factors, preliminary biochemical studies. Samuel L. Jodidi 301-311
kernels, chemical analyses 304-309
shells, chemical analyses 304-309
strains, growing at Holland, Va., on experimental plots, studies 302-303
Peanuts—
acid-hydrolyzable polysaccharides, estimation 308-309
ash content, analyses 304-305
carbohydrate content, analyses 308, 309
crude fiber content, determinations 307
ether-extraction studies 305-306
nitrogen and protein content, analyses 306-307
Pecan trees, roots, carbohydrate and nitrogen content, seasonal variations. C. L. Smith and J. G. Waugh 449-460
Pennsylvania Agricultural Experiment Station, potato growing for foliar diagnosis, experiments 397-414
Peroxidase activity in crown galls and contiguous tissue of tomato, sugar beet, and raspberry 549, 552, 553
PETERSON, W. H., NAGY, R., and RIKER, A. J.: Some Physiological Studies of Crown Gall and Contiguous Tissue 545-555
Phaseolus polystachyus, flowering, inhibition, effect of length of day, experiments 775-776, 779-780, 784-786, 787, 788
Phoenix dactylifera—
infected with *Fusarium moniliforme*, pathologic histology 316

Phoenix dactylifera—Continued. Page
inoculation with *Fusarium moniliforme*, studies 315-316
See also Date palm.
Phoma meliloti, relation to *Stagonospora meliloti* 808
Phosphate rocks, defluorinated, boron in, determination and occurrence. Lewis F. Rader, Jr., and W. L. Hill 901-916
Phosphates, natural, boron in, determination and occurrence. Lewis F. Rader, Jr., and W. L. Hill 901-916
Phosphoric acid content of potato leaf after treatment with complete and imcomplete fertilizers, study 402-414
Photoelectric sorter—
for cotton fibers, description 43-45
for length measurements of fibers of seed cotton. Burt Johnson 41-56
Phytomonas tumefaciens, cause of crown gall 21, 36, 546
Pine—
ponderosa type of California, rate of spread of surface fires. John R. Curry and Wallace L. Fons 239-267
red, wood, moisture movement above fiber saturation point, experiments 161-185
stands—
timber surveys, sampling error 715-735
variability, analysis 718-735
western white—
damage by blister rust infection, studies 558-568
infection by blister rust, relation to damage in individual trees 561-564
tree size, effect on liability to blister rust infection and damage 564-565
young, some representative stands, probable damage by blister rust in, studies. Thomas W. Childs and J. W. Kimmey 557-568
Pinus—
spp., soil-fertility standards for growing 945-951
spp. *See also* under specific name.
Plant growth substance—
and development of crown gall 21-37
effect on tissue inoculated with attenuated strain of *Phytomonas tumefaciens* 33-35
Plants—
cyanogenetic—
preservation for chemical analysis. Reinhold R. Briese and James F. Couch 81-107
specimens, preparation and preservation methods 83-84
flowering in, complete or partial inhibition of, when days are too short or too long. H. A. Allard 775-789
hydrocyanic acid formation and preservation in, studies 81-82
in storage, bacterial decomposition 82
inoculated with crown gall bacteria, responses suggesting presence of growth substance 23-32
susceptible to black ring virus, annotated list 937-939
Polyembryony in cotton, cytological study 155-159
Polyporaceae, wood-destroying, reactions on gallic and tannic media 687-689, 693-695
Pork, muscles, roasted, exterior temperature, effect upon press fluid, shear force, and cooking losses. Alice M. Child and Mary J. Satorius 865-871
Potash content of potato leaf after treatment with complete and incomplete fertilizers, study 402-414
Potassium—
chloride, treatment of potato plants, effects, foliar diagnosis 398-414
fluoride, feeding to grasshoppers, results 426, 427-428
nitrate, effect on growth and physiological alteration of *Azotobacter chroöcoccum*, experiments 217-227
thiocyanate solutions, solubility of casein in, determination 130, 140
Potato—
aphids, feeding habits, determination 324-325, 328-331
Potato—Continued. Page
development, relation to foliar diagnosis. Walter Thomas and Warren B. Mack 397-414
fertilizer treatment, relation to foliar diagnosis. Walter Thomas and Warren B. Mack 397-414
growth rate during early period, relation to final yield of tubers 400-402
leaf roll virus, transmission experiments 319-332
mosaic, transmission experiments 319-332
plants treated with standard and incomplete fertilizers—
appearance of leaves 399
composition of leaves 402-406
development 400-402
development, relation to foliar diagnosis 409-413
tops, development when treated with standard and incomplete fertilizers 400-402
tuber—
membranes, microchemical tests for blue stem disease 387-388
mineral content, microchemical tests for blue stem disease 390-391
storage substances, microchemical tests for blue stem disease 388-389
tissue, autolysis, investigation 434, 438, 439
tubers—
affected with blue stem disease, microchemical studies. L. M. Hill and C. R. Orton 387-392
development when treated with standard and incomplete fertilizers 400-402
viruses, transmission by—
aphids, tests 322-324, 325-327, 331-332
miscellaneous insects, tests 323-324, 327-328, 331-332
vectors, experiments. T. P. Dykstra and W. C. Whitaker 319-334
Potatoes, proteolytic activity in relation to blackening after cooking. A. Frank Ross and W. E. Tottingham 433-441
Preservation, effect upon hydrocyanic acid content of plants, experiments 87-105
Preservatives, miscellaneous, effect on cyanogenetic plants, analysis 89-90, 104-105
Protein—
extract from soybeans, use in food, study. Sybil Woodruff, Evelyn Chambers, and Helen Klaas 737-746
nitrogen, content of feedstuffs, determination 486
substance in soybean—
extraction methods 738-741
physical properties 741-745
Proteolytic activity of potatoes, relation to blackening after cooking. A. Frank Ross and W. E. Tottingham 433-441
Pullets, Rhode Island Red—
clutches, time interval between. F. A. Hays 575-581
egg production, relation to time interval between clutches, studies 575-581
interval between clutches in winter as related to—
hatching-season egg weight and hatchability 579
persistency and annual production 580
size of winter clutch, studies 578
winter egg production, studies 578-579
relation between monthly interval between clutches and monthly interval between eggs, studies 577

Quartz sand, effect on toxicity of sodium selenate to millet, yields, experiments 2-14
QUINBY, J. R., and STEPHENS, J. C.: Linkage of the Q B Gs Group in Sorghum 747-757

Rabbits—
feeding sweetclover to, experiments 146-153
susceptibility to coumarin-treated alfalfa, experiments 149-153
RADER, LEWIS F., JR., and HILL, W. L.: Determination and Occurrence of Boron in Natural Phosphates, Superphosphates, and Defluorinated Phosphate Rocks 901-916

Page

Radish mosaic virus, comparison with cabbage black ring virus 941-942
Raspberry—
aphids, vector in transmission of raspberry mosaic 623-632
red—
crown gall and contiguous tissues, physiological studies 546-553
crown galls, and contiguous tissue, chemical composition 546-552
hybrids, resistance to mosaic infection by *Amphorophora rubi*, experiments .. 604, 630-632
mosaic virus, spread by *Amphorophora rubi*, experiments 624-632
resistance to mosaic vector *Amphorophora rubi* Kalt. Glenn A. Huber and C. D. Schwartze 623-633
varietal resistance to mosaic infection by *Amphorophora rubi*, experiments 626-630, 631-632
varieties, resistance to mosaic infection by *Amphorophora rubi*, experiments 624-630
Ration, dairy, roughage in, color and carotene content, relation to color, carotene content, and vitamin A activity of butterfat. R. E. Hodgson, J. C. Knott, H. K. Murer, and R. R. Graves 513-528
Rations—
livestock, metabolizable energy, determination 507-511
mixed—
digestible nutrients and metabolizable energy. F. W. Christensen and T. H. Hopper 477-512
for livestock, digestibility 493
REES, LOUIS W., and BUCKMAN, STANLEY J.: Moisture Movement in Wood Above the Fiber-Saturation Point 161-187
Refrigeration of plants, effect upon hydrocyanic acid content 85-86
Respiration—
of fungus of genus *Rhizopus*. Selman A. Waksman and J. W. Foster 873-899
poisons, effect on glycolysis by *Rhizopus* pellicles, experiments 891-893, 894-897
Rhizobiosin, growth factor for rhizobium 474
Rhizobium—
growth requirements 462
trace-element requirements, applicability of nutrient-solution purification to study. Robert A. Steinberg 461-476
rifolii, growth, effect of trace elements, cultural sturdies 461, 462-469, 473-475
Rhizopus—
genus of fungus, respiration and lactic acid production. Selman A. Waksman and J. W. Foster 873-899
glycolysis, effects of respiration poisons, experiments 891-893, 894-897
growth—
effect on production of lactic acid 876-887
formation of intermediary products. 893-894, 896
lactic acid production, factors affecting .. 879-893, 894-897
nutrition, investigations 873-898
Riboflavin, in certain milk products, assays. T. H. Jukes and G. A. Richardson 603-610
RICHARDSON, CHARLES H., and SEIFERLE, EDWIN J.: Toxic and Repellent Properties of Some Inorganic and Organic Compounds and Mixtures in Grasshopper Baits 423-432
RICHARDSON, G. A., and JUKES, T. H.: Assays of Riboflavin and the Filtrate Factor in Certain Milk Products 603-610
RIKER, A. J.—
LOCKE, S. B., and DUGGAR, B. M.: Growth Substance and the Development of Crown Gall 21-39
NAGY, R., and PETERSON, W. H.: Some Physiological Studies of Crown Gall and Contiguous Tissue 545-555
Roasts, beef and pork, cooking, effect of exterior temperature upon 865-871
Rocks, defluorinated phosphate, boron in, determination and occurrence. Lewis F. Rader, Jr., and W. L. Hill 901-916

Page

Root knot, resistance in beans and cowpeas in seedling stage, determination method. Keith C. Barron 363-370
Roots, of bearing pecan trees, carbohydrate and nitrogen content, seasonal variations. C. L. Smith and J. G. Waugh 449-460
ROSS, A. FRANK, and TOTTINGHAM, W. E.: Proteolytic Activity in Relation to the Blackening of Potatoes After Cooking 433-441
Rotenone, biological disposition after ingestion by southern armyworm. P. A. Woke 707-712
Roughage in dairy ration, color and carotene content, relation to color, carotene content, and vitamin A activity of butterfat. R. E. Hodgson, J. C. Knott, H. K. Murer, and R. R. Graves 513-528

Saccharum spontaneum, flowering, inhibition, effect of length of day, experiments .. 775-776, 782, 788
Salts—
neutral, of sodium and calcium, effect on carbon and nitrogen of soils. V. P. Sokoloff 203-216
of certain organic acids, solubility of casein in, and its fractionation by means of these acids. Ralph H. McKee and Stephen P. Gould 125-143
Sand-soil mixtures, effects on toxicity of sodium selenate to millet yields 2-7
Sapwood, moisture movement above fiber-saturation point, experiments 161-185
SATORIUS, MARY J., and CHILD, ALICE M.: Effect of Exterior Temperature Upon Press Fluid, Shear Force, and Cooking Losses of Roasted Beef and Pork Muscles .. 865-871
SCHAEFER, CARL W.: Physiological Conditions Which Produce Wing Development in the Pea Aphid 825-841
SCHWARTZE, C. D., and HUBER, GLENN A.: Resistance in the Red Raspberry to the Mosiac Vector *Amphorophora rubi* Kalt .. 623-633
Scolytus—
in white fir, association with brown-staining fungi, further investigations. Ernest Wright 759-773
praeceps, habits and description 759-760
spp., habits and description 759-760
spp., vectors of *Spicaria anomala*, extent of activity 763-764
subscaber, habits and description 760
ventralis, habits and description 759-760
SEIFERLE, EDWIN J., and RICHARDSON, CHARLES H.: Toxic and Repellent Properties of Some Inorganic and Organic Compounds and Mixtures in Grasshopper Baits .. 423-432
Selenium—
content of millet plants, determination, experiments 14-17
toxicity to millet, effect of soil colloids and whole soils, study 1-19
Septogloeum athrix comb. nov., relationship to *Mastigosporium album* var. *athrix* 298
Shasta Experimental Forest, surface fires, spread in ponderosa pine, experiments 241-266
Sheep—
feeding experiments 477-512
metabolism trials with silages, hays, and mixed rations 479-480
SHINN, LEO A., WISEMAN, HERBERT G., KANE, EDWARD A., and CARY, C. A.: The Carotene Content of Market Hays and Corn Silage 635-669
Silage—
corn, carotene content—
Herbert G. Wiseman, Edward A. Kane, Leo A. Shinn, and C. A. Cary 635-669
determination 661-666
in dairy ration, color and carotene content, relation to color, carotene content, and vitamin A activity of butterfat 513-526
Silages—
chemical composition 483-487
digestibility, experiments 487-492

Silages—Continued. Page
digestible nutrients and metabolizable energy. F. W. Christensen and T. H. Hopper 477–512
SIMONDS, AUSTIN O.: The Anatomical Development of *Lepidium draba* 917–928
Simulium nigroparvum—
alimentary tract, histology 446–447
blackfly, digestive tract, morphology. James A. Cox 443–448
gross anatomy 443–445
transmission of blood protozoan disease of turkeys 443
SMITH, C. L., and WAUGH, J. G.: Seasonal Variations in the Carbohydrate and Nitrogen Content of Roots of Bearing Pecan Trees 449–460
SMITH, OLIVER F.: Host-Parasite Relations in Red Clover Plants Resistant and Susceptible to Powdery Mildew, *Erysiphe polygoni* 671–682
SMITH, WILLIAM K., and BRINK, R. A.: Relation of Bitterness to the Toxic Principle in Sweetclover 145–154
Sodium—
benzenesulphonate solutions—
solubility of casein, determination 131, 139–141
use in fractionation of casein, experiments 133–137, 140–141
cymenesulphonate—
recrystallized, solutions of, solubility of casein in, determination 129–130, 139–141
solutions, use in fractionation of casein, experiments 138–139, 140–141
fluoride, influence on glycolysis by *Rhizopus* 891–892
neutral salts, effect on carbon and nitrogen of soils. V. P. Sokoloff 201–216
nitrate treatment of potato plants, effects, foliar diagnosis 398–414
selenate, toxicity to millet, effect of different soil colloids and whole soils on. Philip L. Gile, Hubert W. Lakin, and Horace G. Byers 1–20
Softwood, moisture movement above fiber-saturation point, experiments 161–185
Soil—
colloids, effect on toxicity of sodium selenate to millet. Philip L. Gile, Hubert W. Lakin, and Horace G. Byers 1–20
conditions in forest nurseries, adjustment problems 948–951
fertility standards for growing northern conifers in forest nurseries. S. A. Wilde 945–952
inoculation with tissue from root knot galls of bean, lima bean, and tomato 364–366
organic matter, conservation, effect of neutral salts, experiments 201–215
temperature—
effect upon root knot resistance in beans and cowpeas 364
relation to chlorosis of gardenia. Linus H. Jones 611–621
type, relation to migration of horse strongyle larvae to the surface 339–342, 347
Soils—
carbon content, effect of neutral salts of sodium and calcium. V. P. Sokoloff 201–216
feces buried in, infective horse strongyle larvae developing in, vertical migration, distribution, and survival. John T. Lucker 335–348
nitrogen content, effect of neutral salts of sodium and calcium. V. P. Sokoloff 201–216
virgin forest, nutrient content and associated conditions for growing northern conifers 945–948
whole, effect on toxicity of sodium selenate to millet. Philip L. Gile, Hubert W. Lakin, and Horace G. Byers 1–20
SOKOLOFF, V. P.: Effect of Neutral Salts of Sodium and Calcium on Carbon and Nitrogen of Soils 201–216
Sorghum—
characters, description 750–752
growth curve. A. T. Bartel and J. H. Martin 843–849
Sorghum—Continued. Page
Q B Gs group, linkage. J. C. Stephens and J. R. Quinby 747–757
varieties, preservation for chemical analysis, studies 83–105
Sorghums—
awnless and awned, independent characters, studies 748–749
genetics of, linkage studies 747–755
growth rate, determination 844–846
linkage of Q B and Gs, studies 752–753
Q B Gs group with other single-factor characters in, inheritance studies 753–755
seed color characters, studies 749–750
seed size and early growth, experiments 846–849
Sorter, photoelectric, for length measurements of fibers of seed cotton. Burt Johnson 41–56
Soybean—
protein substance—
appearance and solubility experiments 741–742
in suspension, ability to foam, tests 742–744
physical properties 741–745
value as protein substitute in cookery 744–745
Soybeans, protein—
extract from, use in food, study. Sybil Woodruff, Evelyn Chambers, and Helen Klaas 737–746
extraction methods 738–741
Spicaria—
anomala—
development, comparison with that of *Trichosporium symbioticum* 765–769
development, influence of environment 769–771
identity as staining fungus of white fir 761–763
morphological characteristics 761–763
pathogenicity tests 765
spread by *Scolytus* beetles, extent 763–764
taxonomy 761
isolation from wood surrounding *Scolytus* galleries 760
SPRAGUE, RODERICK: Two Mastigosporium Leaf Spots on Gramineae 287–299
Stagonospora—
leaf spot, relation to root rot of forage legumes. Fred Reuel Jones and J. L. Weimer 791–812
meliloti—
ascigerous form on white sweetclover 808–809
Phoma stage on sweetclover, taxonomy 808, 809
synonymy 804–807
recedens n. comb., occurrence and taxonomy 807–808
root rot fungus—
morphology and development in culture 801–803
morphology and development on host plants 798–801
pathological histology and pathogenicity 794–798
taxonomy 803–809
STANTON, T. R., and COFFMAN, F. A.: Variability in Germination of Freshly Harvested *Avena* 57–72
Starch content of roots of pecan tree, seasonal variation 455–456, 458
Steers, metabolism trials with silages, hays, and mixed rations 479–480
STEINBERG, ROBERT A.—
Applicability of Nutrient-Solution Purification to the Study of Trace-Element Requirements of *Rhizobium* and *Azotobacter* 461–476
Correlation Between Biological Essentiality and Atomic Structure of the Chemical Elements 851–858
The Essentiality of Gallium to Growth and Reproduction of *Aspergillus niger* 569–574
STEPHENS, J. C., and QUINBY, J. R.: Linkage of the Q B Gs Group in Sorghum 747–757
Stock, annual, mosaic virus, comparison with cabbage black ring virus 941–942
Strawberry—
dwarf, occurrence in Massachusetts and North Carolina, study 73–80
plants, occurrence of two distinct strains of nematode *Aphelenchus fragariae* on in United States. J. R. Christie 73–80

Page

Strongyle larvae of horses developing in feces buried in different soils, vertical migration, ibution, and survival. John T. cker ... 335-348
igyles, parasitism in horses, study ... 335-347
BO, CHARLES R., and GAINEY, P. L.: An Apparent Induced Loss of Nitrogen-Fixing Ability in *Azotobacter* ... 217-227
Sudan grass, preservation for chemical analysis, studies ... 83-105
Sugar beet, crown gall—
nd contiguous tissue, chemical composition ... 546-552
and contiguous tissues, physiological studies ... 546-553
Sugar content of roots of pecan trees, seasonal variation ... 455, 458
cane, flowering, inhibition, effect of th of day, experiments ... 775-776, 782, 788
wer silage, feeding to livestock, experiments ... 477-482, 484, 489-490, 496-497, 506-511
superphosphate treatment of potato plants, effects, foliar diagnosis ... 398-414
Superphosphates, boron in, determination and occurrence. Lewis F. Rader, Jr., and W. L. Hill ... 901-916
Sweetclover—
bitterness, relation to toxic principle. William K. Smith and R. A. Brink ... 145-154
disease—
description ... 145-146
susceptibility of rabbits, experiments ... 146-153
Hubam, spoiled, feeding to rabbits, experiments ... 147-148, 152
silage, feeding to livestock, experiments ... 477-487, 493, 500-510
stagonospora leaf spot and root rot, cultural studies ... 791-810
stems, feeding to livestock, experiments ... 477-481, 482, 491-493
toxic principle, relation to bitterness. William K. Smith and R. A. Brink ... 145-154

Tannic acid medium, reactions of wood-decaying fungi on, differentiation. Ross W. Davidson, W. A. Campbell, and Dorothy J. Blaisdell ... 683-695
Temperature—
effect on—
development of *Trichosporium symbioticum* and *Spicaria anomala*, tests ... 765-766
growth and lactic acid production by *Rhizopus* ... 881-882, 894-897
growth rate of apple ... 110, 113, 116-119, 122
hydrocyanic acid in stored plants, experiments ... 85-86
root knot resistance in beans and cowpeas ... 364
environmental, effect on mortality, rate of growth, and utilization of food energy in White Leghorn chicks. C. F. Winchester and M. Kleiber ... 529-544
exterior, effect upon press fluid, shear force, and cooking losses of roasted beef and pork muscles. Alice M. Child and Mary J. Satorius ... 865-871
soil, relation to chlorosis of gardenia. Linus H. Jones ... 611-621
Tetrastichus—
sp. "Y", adult, comparison with adult of *T. xanthomelaenae* ... 862
xanthomelaenae and *Tetrastichus* sp., hymenopterous egg parasite of elm leaf beetle, laboratory studies. P. A. Berry ... 859-863
Thallous sulphate, feeding to grasshoppers, results ... 426, 427-428
Thelephoraceae, wood-destroying, reactions on gallic and tannic media ... 686
Thespesia spp., leaf and stem anatomy, investigations ... 270-283
THOMAS, H. REX, TOMPKINS, C. M., and GARDNER, M. W.: Black Ring, a Virus Disease of Cabbage and Other Crucifers ... 929-943
THOMAS, WALTER, and MACK, WARREN B.: Foliar Diagnosis in Relation to Development and Fertilizer Treatment of the Potato ... 397-414

Page

Timber—
stands, variability, analysis ... 718-735
surveys, sampling, error in. A. A. Hasel ... 713-736
Time—
element, in spread of fire in ponderosa pine stands, tests ... 241, 242-243, 247-249, 254-256, 260-266
interval between clutches in Rhode Island Red pullets. F. A. Hays ... 575-581
Tiphia popilliavora—
life cycle ... 380
population—
and sex, influence of Japanese beetle instar on. M. H. Brunson ... 379-386
effect of Japanese beetle abundance and instars ... 384, 385-386
effect of periodic fluctuation in number of third-instar hosts ... 384-386
preference for instar of host larvae for oviposition ... 380, 381, 385
progeny, sex of, relation to host instars ... 381-383, 385
rearing methods ... 380
Toluene, effect on hydrocyanic acid content of plants, experiments ... 89-90
Tomato—
cambial stimulation by inoculations of crown gall, experiments ... 27-29
crown gall and contiguous tissue—
chemical composition ... 546-552
growth substance, role in development of crown gall, experiments ... 23-35
physiological studies ... 546-553
stems, inoculation with beta-indole-acetic acid, experiments ... 34-35
tissue inoculated with crown gall bacteria, determination of growth substance in ... 32
TOMPKINS, C. M.—
A Mosaic Disease of Turnip ... 589-602
GARDNER, M. W., and THOMAS, H. REX: Black Ring, a Virus Disease of Cabbage and Other Crucifers ... 929-943
TOTTINGHAM, W. E., and ROSS, A. FRANK: Proteolytic Activity in Relation to the Blackening of Potatoes After Cooking ... 433-441
Trace-element—
requirements of—
Aspergillus niger, tests ... 569-573
rhizobium and azotobacter, applicability of nutrient-solution purification to study. Robert A. Steinberg ... 461-476
See also Metals, heavy.
Trichosporium symbioticum—
development—
comparison with that of *Spicaria anomala* ... 765-769
influence of environment ... 769-771
isolation from wood surrounding *Scolytus* galleries ... 760-761
rate of stain, comparison with *Spicaria anomala*, tests ... 759, 760, 763, 764, 765-771
Trifolium spp., stagonospora leaf spot and root rot fungi, cultural studies ... 791-810
Tryptophane in blackening cooked potatoes, study ... 435, 436, 438, 439
Turnip mosaic—
disease—
C. M. Tompkins ... 589-602
symptoms ... 591-593
virus—
comparison with cabbage black ring virus ... 941-942
experimental host range ... 594-597, 600
from New York, comparison with other crucifer viruses ... 598-599
from New York, description ... 598
properties and description ... 597-598
transmission by aphids, experiments ... 593-594
Tyrosinase activity in crown galls and contiguous tissue of tomato, sugar beet, and red raspberry ... 551, 552-553
Tyrosine, in blackening cooked potatoes, study ... 435, 436, 438, 439-440

United States, strawberry plants, two distinct strains of the nematode *Aphelenchoides fragariae* occurring in. J. R. Christie ... 73-80

Page

Vapor, movement in wood blocks, experiments ... 179–183
Vectors, transmission of potato viruses, experiments. T. P. Dykstra and W. C. Whitaker ... 319–334
VERNER, LEIF: Histology of Apple Fruit Tissue in Relation to Cracking ... 813–824
Viruses. *See* under s
Vitamin A—
activity of butterf[illegible] color and carotene conten[illegible] in dairy ration. R. E. H[illegible] C. Knott, H. K. Murer, and [illegible]es ... 513
in cattle feeds, relation [illegible]e content, experiments ... 63[illegible]
value of butterfat, determina[illegible] ... 515–516, 52[illegible]
Vitamin D potency of stemmy and leafy portions of alfalfa hay, comparison. G. C. Wallis ... 393–396

WAKSMAN, SELMAN A., and FOSTER, J. W.: Respiration and Lactic Acid Production by a Fungus of the Genus *Rhizopus* ... 873–899
WALLIS, G. C.: A Comparison of the Vitamin D Potency of the Stemmy and Leafy Portions of Alfalfa Hay ... 393–396
Washington, Wenatchee Valley, growth rate of Winesap apples, studies ... 109–123
WAUGH, J. G., and SMITH, C. L.: Seasonal Variations in the Carbohydrate and Nitrogen Content of Roots of Bearing Pecan Trees ... 449–460
WEBBER, IRMA E.: Anatomy of the Leaf and Stem of *Gossypium* ... 269–286
WEBBER, J. M.: Cytology of Twin Cotton Plants ... 155–160
WEIMER, J. L., and JONES, FRED REUEL: Stagonospora Leaf Spot and Root Rot of Forage Legumes ... 791–812
Wheat—
germination variability when freshly harvested. F. A. Coffman and T. R. Stanton ... 57–72
plants, tolerance to sodium selenate, effects of soils ... 17
WHITAKER, W. C., and DYKSTRA, T. P.: Experiments on the Transmission of Potato Viruses by Vectors ... 319–334
WILDE, S. A.: Soil-Fertility Standards for Growing Northern Conifers in Forest Nurseries ... 945–952
WINCHESTER, C. F., and KLEIBER, M.: The Effect of Environmental Temperature on Mortality, Rate of Growth, and Utilization of Food Energy in White Leghorn Chicks ... 529–544
Wind velocity, effect on spread of fire in ponderosa pine stands, tests ... 242, 243, 245–252, 254–25[illegible]
Wireworm—
body, entrance of arsenic, discussion of method ...
Pacific coast, prevention of entrance of undesirable substances into digestive tract ...
Wireworms, reactions to—
arsenical solutions, tests ... 231, [illegible]
[illegible]ls, Charles E. Woodworth ...
[illegible]ch pellets containing—
[illegible]cals, tests ... 229–230,
[illegible]senicals, tests ...
[illegible]AN, HERBERT G., KANE, EDWA[illegible]
[illegible]HINN, LEO A., and CARY, C. A.: T[illegible]
[illegible]otene Content of Market Hays a[illegible]
[illegible]n Silage ...
[illegible]E, P. A.: The Biological Disposition [illegible] Rotenone After Ingestion by the Southern Armyworm ...
Wood—
blocks—
evaporation rate, studies ... 175–1[illegible]
moisture loss, rate, studies ... 165–174, 177, 178, 180
water-pressure movement, rates ... 178, 179
combustion, theoretical aspects ... 249–250
decaying fungi, differentiation by their reactions on gallic or tannic acid medium. Ross W. Davidson, W. A. Campbell, and Dorothy J. Blaisdell ... 683–695
moisture movement—
above fiber-saturation point. Louis W. Rees and Stanley J. Buckman ... 161–187
factors influencing by different mechanisms ... 174–175
WOODRUFF, SYBIL, CHAMBERS, EVELYN, and KLAAS, HELEN: A Study of Protein Extract From Soybeans With Reference to Its Use in Food ... 737–746
Woods, moisture movement to surface, methods and rates ... 177, 179–183
WOODWORTH, CHARLES E.: The Reactions of Wireworms to Arsenicals ... 229–238
WRIGHT, ERNEST: Further Investigations of Brown-Staining Fungi Associated With Engraver Beetles (*Scolytus*) in White Fir ...

Zinc—
arsenite, feeding to grasshoppers, results ... 427–42[illegible]
influence on growth of and lactic acid production by *Rhizopus*, experiments ... 883–887, 896–897
phosphide, feeding to grasshoppers, results ... 426, 427–428